Investigative Study on the Interaction and Applications of Plasma Activated Water (PAW)

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Abstract. Cold plasma is a budding technology that can be used as a nonthermal disinfectant and for surface modification which is free of chemicals and is environmentally friendly. The treatment of water with plasma, producing plasma activated water (PAW), generates an acidic condition resulting in the formation of reactive oxygen species (ROS), reactive nitrogen species (RNS), and creates change in the redox potential as well as conductivity. Consequently, the chemical composition of water and PAW differ from each other and can now be employed as a substitute for disinfection against microbes. The various sources of plasma used for PAW production, its physical and chemical properties and its prospective uses are reviewed in this paper. Particularly, the physiochemical properties of PAW will be discussed in the context of its acidity, conductivity, the amount of ROS and RNS, as well as its redox potential. Since the results are microbial in nature, the microbial disinfection with the use of PAW will also be reviewed. Lastly, the usage of PAW to enhance agricultural methods, such as its effect in plant growth and stimulating seed germination, is also discussed. It can be interpreted that PAW synergistically disinfects food as well as enhances the growth of seedlings. The boost in plant growth conceivably be mainly due to the increase in concentration of nitrate and nitrite ions in PAW. Therefore, in addition to the antimicrobial action of PAW, submerging seedlings in PAW supplements the germination of seeds and plant growth. This could possibly help fight against the drought stress and improve the yield of crops.

Keywords. Plasma Activated Water (PAW); Microbial disinfection; Reactive nitrogen species; Reactive oxygen species.

1. Introduction
It is estimated that within the next 30 years the population of earth will reach to about 10 billion, which implies the need of a revolutionized approach to food processing and production to meet the increasing global demands. The emergence of pathogens poses a challenge to produce high quality safe food to meet the increasing demand. For example, the outbreak of Shiga-toxin which produced Escherichia coli bacteria (STEC) serotype O104:H4 proved to be fatal to 46 people and resulted in a large outbreak of diarrhoea and Haemolytic uremic syndrome [1]. Consumers now increasingly demand for food that is least processed and contains minimal amount of preservatives and better

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quality of food, a solution to the existing methods of food preservation could be nonthermal processing techniques which decreases the unfavourable effects to the nutritional quality of food. Irradiation, UV light processing, high pressure processing and ozonation are nonthermal technologies that are currently being applied to increase the shelf-life of food [2]. Pulsed electric fields and ultrasounds are other examples of nonthermal processing technologies applied on fruits and vegetables to extract its bioactive compounds.

Atmospheric cold plasma is a reasonably new technology that is being used as a nonthermal decontamination of food. Plasma, a fourth state of matter consists of negatively and positively charged ions, electrons, atoms in their excited and neutral state, ground state and excited state molecules and UV photons[3]. Based on their thermodynamic temperature equilibrium, plasma is further subdivided into thermal (hot) and nonthermal (cold) plasma temperature of which does not exceed beyond 60 °C. Corona discharges, dielectric barrier discharges and plasma jets are some of the most commonly used sources for plasma in food application technologies. Researchers and scientist have applied the use of cold plasma in enhancement of seed germination, in decontamination of food, modification of starch, to improve the quality of rice and enzyme inactivation [4]. The application of gas plasma on food surfaces to obtain microbial inactivation has led to loss of flavour, texture, colour, and degradation of the overall general appearance. PAW has been designed such that it could eliminate the traditional methods and avoid its adverse effects. Studies have shown promising results for efficient control of bacterial growth and inactivation with the use of PAW. However, for this technology to be considered for use in large scale industrial applications and to replace the pre-existing sanitizing methods, further studies need to be conducted paying particular attention on the Life Cycle Assessment. This review will present and introduction to PAW, plasma sources for PAW production, its physiochemical properties, and the possible use of it in food and agriculture. It will also be presenting potential alternates for the sanitation and disinfection of food to improve current agricultural practices.

2. Chemistry and production of PAW
The type of gases and liquids used to produce PAW, will determine the type and amount of reactive species in it. The formation of these reactive species depends on the voltage, treatment time, generation mode and the chemical environment. Furthermore, the nature of electrodes, and the distance between the liquids and plasma plume should also be considered. For instance, when the parent molecules used in PAW production are water, oxygen and nitrogen, the resulting outcome consists of variety of primary species, which then further react to form secondary species [5, 6]. These chemical reactions are those that culminate ROS and RNS in PAW that has antimicrobial properties. The two methods of PAW production include contact of plasma streaming with the water and inducing plasma directly into water, consequently the chemistry and resulting products of PAW differ from the one produced above water to the one produced in the liquids.

Figure 1 provides a schematic illustration of a typical experimental setup utilised for generating PAW while examining the PAW chemistry evolution along with the characteristics of the current and voltage in the plasma jet. This experimental configuration was used by [7] for generating PAW, where an in-situ UV absorption spectroscopy was also utilised for measuring the production of O₂, NO₃⁻, NO₂⁻, and H₂O₂ in deionized water. In addition, a current monitor along with a voltage probe was used. In terms of the plasma jet, this has been used in several other research studies as well [8, 9, 10]. The outcome of their [7] work showed that the concentrations of O₂, NO₃⁻, NO₂⁻, and H₂O₂ produced in deionized water with a Monopolar direct current pulse driven non thermal atmospheric pressure plasma jet are dependent on the plasma jet’s pulse polarity changes as well as the pulse width. In addition, the results of their study revealed that when a positive pulse was used to operate the plasma jet, an increased concentration of O₂, NO₃⁻ , NO₂⁻, as well as H₂O₂ were produced at shorter pulse widths. However, for the plasma jet with a negative pulse, an opposite trend was observed. Nevertheless, for both the plasma jets observed, the concentration of O₂ rose with an increase in the pulse width. On the whole, the study outcomes indicated the possibility of regulating the interactions of PAW generated with the aid of a Monopolar direct current pulse driven non thermal atmospheric pressure Helium plasma jet through the adjustment of the pulse width and pulse polarity.
3. Physiochemical characteristics of PAW

3.1. Discussion on the pH of PAW

The chemical species in the plasma react with the water leading to acidification of the PAW as measured on the pH scale. The pH of PAW is inversely proportional to the treatment time; longer treatment time leads to in formation of strong acids. The patented PAW developed by [11] states that for the PAW production with a suitable combination of both thermal and nonthermal plasma results in a controlled pH. The low pH of PAW is caused by the formation of nitric acid, hydrogen peroxide and peroxynitrous acid from the acidification of aqueous liquids through the plasma, as reported by [12]. These newly formed species are attributed to the antimicrobial activity of PAW. A steep drop in the pH was observed by [13] and [14] at the initial phase of plasma treatment before reaching a steady range showing almost no change at all. The type of feed gas and reactor causes variations in the pH of the generated PAW. Moreover, it has been noted that storage conditions bring about a change in the pH of PAW. pH was seen to be slightly lower in PAW generated from air plasma as compared to PAW generated from oxygen which showed a slight increase. [15] noticed the pH of PAW stored for about 70 hours to have decreased. [16] examined the effect of storage temperatures on the pH of PAW, it was determined that no significant change in pH values occurred over the course of 30 days. A similarity in the pKa value of PAW after activation with pKa of nitrous acid was also found.
3.2. The redox potential of PAW
A speedy assessment of the disinfection potential of PAW can be measured with a single value—the oxidation-reduction potential (ORP), which is a measure of the ability of the solution to oxidise or reduce another substance [17]. Microbial inactivation is affected by the ORP, damaging the microbial cellular membrane and its defence mechanisms [18]. Among the many ROS formed in PAW, the main species involved as the oxidant and reductant is the hydrogen peroxide [19]. An increase in ORP is ensued by the addition of oxidizing chemicals as recorded by [20]. PAW produced inside water had a higher ORP value compared to PAW produced above the water surface as observed by [21]. As seen with pH, a difference in the storage temperatures of PAW had no notable change in ORP value whereas different storage times did result in a decrease of ORP values [16]. Finally, the authors [13] reported a proportional relationship between the ORP values and the antimicrobial potential; higher the ORP value, stronger the oxidising capability, higher the antimicrobial property.

3.3. Conductivity of PAW
The ease of which electricity can flow through water is a measure of its conductivity. The resulting ROS and RNS from plasma activation of water provides an increase in conductivity of PAW, and the reactive species and ions formed during the plasma generation changes its conductivity. [13] observed the conductivity of distilled water to have increased after activation with argon and oxygen gas, which is due to the formation of ions. Moreover, the conductivity of PAW is lower when generated over the water surface compared to when generated directly inside the water. A linear increase in conductivity was observed when using atmospheric plasma jet to produce PAW in a study conducted by [14]. Intense radiations and higher electron density were observed in the discharge as a result of the increased conductivity [22]. Nitrogen oxides have been attributed to increase the conductivity, whereas, working without nitrogen gases, the water lacks nitrogen oxides and the conductivity is caused due to the H3O+ ions in the water [19]. A research conducted by [23] on the effect of applied voltage and conductivity of water on the intensity of UV radiation. With the increasing conductivity of the water, an increase in UV radiation was seen. The conductivity of PAW differs with the plasma devices, and carrier gases used.

3.4. H2O2 in PAW
The formation of the ROS, H2O2, in PAW plays an important role in its antimicrobial properties. Assayed using iodometric titration, titanium oxalate, peroxidase enzyme kit and permanganate solution, its formation and importance can be evaluated. Along with superoxide anion, the H2O2 concentration in the acidic medium grants to the most significant part of the oxidation properties of PAW [24]. The electronic impact of water molecules giving rise to OH radicals get converted to H2O2, which is later transported into the treated liquids. [25] conducted a study to investigate the factors that determine the concentration of H2O2. They concluded that discharge duration and applied voltage increases the formation of H2O2. [23] demonstrates that the conductivity of the water determines the concentration of H2O2 after discharge.

3.5. Nitrites and nitrates ion concentration in PAW
In recent years, the antimicrobial activity of RNS has piqued the interests of many researchers. The detection of nitrites and nitrates verifies the presence of RNS. Extended antimicrobial properties of PAW have been reported due to the nitrate ions formed as the secondary products [16]. The nitrite ion reacts with the hydrogen peroxide forms peroxynitritre which imparts a significant antimicrobial activity. The concentration of nitrite and nitrate ions decrease with the increase in storage time, similar to ROS concentrations. Nitrite ions form as a result of the dissociated N2 and O2 (nitrogen oxides) in the air plasma reacting with the water, which then reacts with the hydrogen peroxide molecule to form nitrate ions. [6] suggested formula for the formation of nitrate is as follows:

\[
\text{NO}_2^- + \text{H}_2\text{O}_2 + \text{H}^+ \rightarrow \text{NO}_3^- + \text{H}_2\text{O} + \text{H}^+ \tag{1}
\]

A difference in concentration of nitrates ad nitrites is observed by discharging plasma above the water surface and directly into the water, the concentration of the ions being higher in the latter method [21]. Experiments have been conducted to study the effects of these nitrogen oxide ions in plant growth
[26]. Hydroxyl and superoxide radicals are secondary oxidants produced by the nitrates and nitrites acting as indirect photosensitizers in the presence of UV radiations [27].

In a recent study by [28], the authors aimed to evaluate the antiseptic characteristics of PAW under biomimetic terms. To do so, the PAW fluid prepared had the following physiochemical properties:

| Property       | Value          |
|----------------|----------------|
| pH             | 2.78 ± 0.12    |
| Oxidation Reduction Potential | + 1.06 V |
| Conductivity   | 446 ± 25 μS/cm |
| NO₂⁻           | 192 ± 10 mg/L  |
| NO₃⁻           | 1550 ± 95 mg/L |
| H₂O₂⁻          | 2.6 ± 0.12 mg/L |
| O₃⁻            | 1.08 ± 0.07 mg/L |

The study [28] concluded that the prepared PAW could be successfully utilised for disinfection purposes.

4. Applications of PAW

The application of using cold plasma directly on food surfaces playing a crucial role in the disinfection has been well established. Some of the important factors determining the microbial inactivation capacity are the working gases and the applied voltage, as seen in several studies. One of the most important responsibility of the food safety programs is to ensure the washing, disinfecting, and enhancing shelf life of the fresh produce before reaching the consumers. The application of PAW is gaining popularity in this regard as potential disinfecting and antimicrobial solution. Currently, the use of high oxidation reduction potential (ORP) water that is obtained by adding chemicals to water can be substituted by PAW. Plasma activated water has also demonstrated enhancement in seed germination and plant growth which could be its other potential use. The reactive species formed in PAW plays a significant role in the germination of seeds and the development of plants, as reported by [29]. [26] suggests the change of water using nonthermal plasma and using it for agricultural activity can enhance the plant growth rate, product nutritional yield and quality.

4.1. Microbial inactivation by PAW

Illustrated in Table 1 is the log reduction of microbial count after the treatment with PAW on planktonic cells, surface attached cells, and adherent cells on food surfaces. ROS and RNS species formed during PAW generation are responsible for the inactivation of microbes. ORP and low pH work synergistically in the formation of these chemical species in PAW [20]. The mechanism of microbial inactivation and the reaction products are drastically changed in the presence of humid air conditions or if contacted by water as vapour or liquid, reported by [30]. UV radiations, strong electrical fields and shock waves are the physical parameters that give PAW its antimicrobial activity in addition to the chemical species generated when using underwater streamer discharge [31]. Therefore, these parameters need to be investigated in a deeper manner to understand their theoretical physics.

The latest applications of PAW on fresh produce conducted by [13] reported a decrement in bacterial count after 15 minutes of treatment with the water. Another study that used cold atmospheric gas plasma for treatment 15 minutes reported only a slight decrease in bacterial count, demonstrating a higher inactivation percentage obtained by PAW [32]. The surface topography of the produce could be a factor that caused the inactivation inefficiencies. The grooves and ridges of produce could prevent the cold gas plasma from reaching all surfaces, whereas this problem is avoided by soaking in PAW.
which assists the reactive species to inactivate the microbes. [14] studied the effect of PAW generated by a plasma plume over the water surface on the microbial inactivation. Microbial cell death occurred due to reactive species accumulating around the cell and breached through the cell membranes. Similarly, another study by [21] noticed about three times more reductions in bacterial count in logarithmic scale when using PAW generated by plasma discharge directly into the water. This increase in efficiency could be due to the synergistic effect granted by the physical activities like shock waves and electric fields generated in the water [23]. Investigating the effect of PAW on Erwinia spp. created using gliding arc plasma showed complete inactivation [33]. Likewise, [34] noticed inactivation of almost all Hafnia alvei with PAW using gliding arc plasma and stated that the efficiency of microbial inactivation differs when using PAW generated with the plasma plume than without. A small number of studies have reported that the efficiency of microbial inactivation when plasma is applied through or directly on PAW is to a lesser degree on fungal cells as compared to on bacterial cells. This is probably due to the complexity of cell structures of eukaryotes and prokaryotes. Fungal cells tend to have a stronger defence system that protects the cells from oxidative stress imposed by the plasma treatment [35]. As for virus cells, the effect of PAW is yet to be studied but some reports are available with regards to cold gas application [36, 37].

### Table 1. Microbial count after treatment with PAW (Adapted from [38]).

| Microorganism | Mode of activation | Activation time (min) | Storage period (day) | Treatment time (min) | Inactivation (in Log_{10}CFU/mL or CFU/μL) | Form of cells | Reference |
|---------------|-------------------|-----------------------|----------------------|---------------------|----------------------------------------|--------------|----------|
| K. cell       | Hydro discharge over the water surface | 20                    | -                    | 15                  | 5.6                                    | Flavobacterium | Taylor et al. (2011) |
| Aerobic bacteria | Hydro discharge over the water surface | 15                    | -                    | 0.5, 1, 1.5         | 1, 2.5                                  | Flavobacterium | Shainey et al. (2012) |
| Aerobic bacteria | Hydro discharge over the water surface | 7                     | -                    | 15                  | 7                                      | Flavobacterium | Odervege et al. (2009) |
| S. aureus     | Hydro discharge inside the water | 39                    | 1, 3, 7, 15, 30      | 20                  | 6.2                                    | Hydro discharge | Zhang et al. (2016) |
| S. aureus     | Hydro discharge inside the water | 10                    | 9                    | 5, 10, 15           | 6.2                                    | Surface attached cells | Zhang et al. (2016) |
| R. albertii   | Hydro discharge over the water surface | 5                     | -                    | 10, 30, 30          | 1.84, 1.36, 5.36                         | Hydro discharge | Zhang et al. (2016) |
| R. albertii   | Hydro discharge over the water surface | 5                     | -                    | 10, 30, 30          | 3.5, 3.4, 5.5, 5.0                        | Surface attached cells | Zhang et al. (2016) |
| R. albertii   | Hydro discharge inside the water | 5                     | -                    | 20                  | 3, 6, 12, 17, 69                         | Hydro discharge | Zhang et al. (2016) |
| R. albertii   | Hydro discharge inside the water | 39                    | 5, 10, 15, 30        | 20                  | 6, 8, 57, 25                            | Adherent cells | Kocberg-Yuval et al. (2009) |
| R. albertii   | Hydro discharge inside the water | 20                    | -                    | 2, 4, 6             | 3, 2, 4, 4                              | Adherent cells | Kocberg-Yuval et al. (2009) |

#### 4.1.1. Microbial inactivation: Role of ROS

Several ROS species are produced during PAW generation, among which O₃ and the hydroxyl radicals are the most potent in their antimicrobial activity. The outermost membrane of the microbial cells is targeted by the hydroxyl radicals. The hydroxyl radicals interact with the lipid membrane, peroxidizing it by removing a hydrogen atom from the unsaturated fatty acid resulting in malondialdehyde (MDA) as a reaction end product. This MDA is used to measure the extent of lipid oxidation [30]. DNA damage and as a result, cell death is caused by the formation of MDA [39, 40]. The hydrogen peroxide and hydroxyl radical have the highest potential among the ROS to break the intramolecular bonds of the lipid layer leading to structural damage of the bacterial cell wall [41].

#### 4.1.2. Microbial inactivation: Role of RNS

Among the RNS species produced during PAW generation, nitric oxide radical and its derivatives such as peroxynitrates, nitrites and nitrates are the most potent. By lowering the pH of PAW, the RNS species increase the microbial susceptibility. Acidification of PAW by the formation of HNOOH, HNO₃, and HNO₂ is responsible for the microbial inactivation. The peroxynitrite ions formed in PAW are extremely strong oxidizers causing microbial inactivation. Acidified plasma processed liquids have a different efficiency levels as compared to PAW, reported by [42]. The synergistic effect, which is available in PAW by the RNS, ROS species and other physical variables is not the case for acidified water, hence a difference in efficiency is observed [34]. Change in conductivity of PAW can be used to detect the concentration of peroxynitrite in the solution. Even through the half-life is peroxynitrates is short, it is enough to cause microbial inactivation as reported by [19]. The peroxynitrite ions initiate a lipid peroxidation reaction by interacting with the lipid
membrane, eventually causing cell death. The microbial cells undergo oxidative stress, cytotoxic and mutagenic damage [43].

4.1.3. Inactivation of microbes by physical factors
Physical factors such as ORP, pH, electric fields, and UV radiation are responsible for the inactivation of microbes in PAW, as demonstrated in Figure 2. The decrease in pH of PAW caused due to the presence of reactive species, greatly affects the microbes as they lack an internal mechanism to adjust the pH [44]. Bacteria cannot survive a pH below 3.7 but yeast and mold tend to be more resistant against acidic pH. ROS and RNS are direct measures of the pH and ORP, mainly dependent on the feed gas, activation time and distance from the surface of the liquid. UV radiations dimerize thymine bases of the bacterial DNA strands [45]. Almost half of the microbial inactivation was caused due to UV radiations formed as a result of plasma discharge into the water. Inactivation of microbes can also be brought about by generating shockwaves in PAW by the formation of cavitation bubbles. Electroporation of cell membranes by high intensity electrical fields also prove to be fatal for microbes, the permeability of the cells increases, and the reactive species gain easy pass into the cells causing oxidative damage to the cells [19].

4.2. Enhancing seed germination and stimulating plant growth
Loss in crop yields is seen due to the loss of seed survivability, low percentage of seeds that undergo germination and longer germination times [46]. [47] studied the effects of cold plasma on seed germination and found that the growth was enhanced during drought stress conditions. At the present time, the applications of PAW for seed enhancement is narrow. [48] studied the effects of the reactive species on seed germinations besides the hormones, and found that the ROS, such as hydroxyl radical, superoxide, and atomic oxygen take part in multiple signaling pathways that determine seed germination. [49] states that the hormones that causes seeds to remain dormant can be decreased by the hydrogen peroxide in PAW. [50] reports that the nitrate species in PAW led to nitrate stimulated seed germination, by altering the phytochrome activity of the seed. Since plants rely heavily on nitrogen fixated by the soil for growth, nitrate rich PAW can enhance plant growth. An experiment
conducted by [51] on the effect of PAW on plant growth found that the plant group that received the treated water had an increased height, with bigger leaves than the control group.

5. **Drawbacks of PAW**
The intricacy of the atmospheric plasma devices, the differences in model designs and device operations result in varying reaction compounds with varying mechanisms. Controlling the reactions and the complexity of gas plasma presents itself as a complicated problem. One of the significant limitations in the produced PAW is the short half life of the reactive species OH, \(^1\)O\(_2\). Special attention should be paid to the different biological effects of PAW produced by two different methods, as there are major differences in PAW generated by a direct discharge in the water as compared to that generated over the surface of the water. Comprehensive diagnostic tools need to be employed to study the antimicrobial efficiency of the plasma and the PAW. It is hard to differentiate which physical or chemical property of PAW is responsible for its antimicrobial property. The mechanisms and reaction processes of the various oxidizers formed in PAW that execute antimicrobial activity is not known. The source which causes a decrease in pH giving PAW its antimicrobial property is still not fully understood. The carrier gas used determines the end reaction products formed in the plasma and using inert gases increases the cost of operation. A clear link between the molecular compounds that are formed in the PAW and the properties of plasma have yet to be established.

6. **Potential of PAW in terms of its future aspects**
A great deal of effort is needed to understand the underlying principles of PAW production and its mechanism of action at a molecular level, these challenges need to be overcome to increase food production and enhance food quality and safety. To overcome these challenges, it is essential to identify the reaction intermediates and to determine what the reaction end products will be after the treatment. A wide range of applications can be developed if the toxicity of the different reaction compounds can be determined. A careful assessment and evaluation of the various ROS and RNS species, and the complexity of the plasma chemistry must be done. Using PAW for hydroponic applications could be exploited for its plant growth enhancement ability. One of the major obstacles for the applications of PAW is to get it approved as a generally regarded as safe (GRAS) treatment method from the different government regulatory bodies. This technology will only be successful if it proves itself as a durable, functional, and productive in large scale industrial operations in the future. Overall, PAW is attaining great attention in several fields such as the biomedical applications, due to its potential bactericidal properties. It is anticipated that the bactericidal characteristics of PAW are initiated because of the reactive nitrogen species and the reactive oxygen species. However, for improving the efficacies of the PAW, understanding the mechanism of plasma jet is important. This would require comprehending the chemistry of key reactive species in water for short- and long-term reactions. Once command on the reactive nitrogen and reactive oxygen species is obtained, amending the optimal chemistries of these species for certain applications would be possible. PAW can be used in various disciplines, such as decontamination foods, agriculture, medicinal and biological fields [7],[13] reported that PAW has outstanding antibacterial abilities and has the potential for the inactivation of a specific bacteria found inoculated on strawberries. Their study also concluded that the use of PAW does not result in any major changes in the pH, firmness and colour of the treated strawberries. Hence, it appears that PAW is a promising alternate to the conventional disinfectants utilised in the fresh produce industries.

7. **Concluding remarks**
Many investigations have reported on the antimicrobial properties of PAW. Discharging plasma directly into the water or discharging over the water surface has shown antimicrobial inactivation activity. This article discussed the fundamental properties, and the antimicrobial activity of PAW. Microbial cell inactivation is achieved chemically by the reactive species formed in PAW reacting with the cell organelles and cell wall and membrane. Structural damage achieved by the use of UV radiations also explains microbial inactivation during PAW generation. The adverse effects of traditional disinfection methods to increase shelf life can be replaced with the use of PAW and fresh
produce without the loss of flavour, texture and colour can be obtained. PAW is drawing attention due to its effectiveness for stimulation of plant growth and enhancing seed germination. Significant improvements can be seen by treating seeds with PAW, which is rich in nitrogen species serving as nutrients, it also exhibits ability to inhibit hormones that causes seed dormancy. The various applications of PAW as a decontaminant, disinfection and an ecofriendly stimulant for enhancing seed germination and plant growth demonstrates a similar activity as shown by cold plasma, therefore, PAW is the next generation ecofriendly disinfectant solution and plant seed stimulant.

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