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Impact of Plasma Treatment on Lady's Finger Seeds for Germination and its Growth

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ABSTRACT

Atmospheric pressure plasma has emerged as a promising technology in various fields, including plasma medicine, agriculture, food safety and storage, and food manufacturing. Plasma treatment has proven to be beneficial in plasma agriculture, as it enhances seed germination, plant growth, and resilience against both abiotic and biotic stresses. Additionally, it can aid in the removal of pesticides, increase biomass, and improve yield. However, the molecular mechanisms underlying the effects of plasma treatment on seed germination and plant growth are not yet fully understood. Therefore, in this study, the authors aimed to investigate the physical and chemical properties of Lady's finger seeds treated with cold plasma, characterized by 2.86 W dissipative power at 7 kV in an argon environment and electron density of 4.53×10^{10} cm^{-3} under atmospheric conditions. The FTIR technique was used to identify the functional groups present in the seed before and after treatment, revealing the presence of C-H, C=O, C-O, and N-O functional groups, as indicated by their corresponding absorption peaks at different wave numbers. The stretching of bonds between elements of the functional groups was observed based on transmittance. Moreover, the treated seeds were germinated, and the physical growth of different parts (root, shoot, and leaf) was measured. The results showed that the growth rate of different parts was higher in the treated seeds than in the untreated.

Keywords: Plasma, Seed germination, Lady's Finger seeds, FTIR technique, Argon environment, Functional groups, Transmittance, Physical growth.

1. INTRODUCTION

1.1 Effect of Plasma on Seed Germination

Seeds are an important part of the plant's life cycle. It harbors the genetic information essential for the growth and development of new plants. Furthermore, seeds are crucial for plant survival, propagation, and dispersal. Although essential for seed survival, dormancy can hinder seed germination even under favorable conditions. Cold plasma treatment is a promising technique for this purpose, as it activates the plant's natural defense mechanisms and stimulates seed growth and development. The treatment of seeds has been found to increase seed germination rates, improve plant growth, and enhance resistance to biotic and abiotic stresses. Fig. 1 illustrates the initiation of seed germination through cold plasma treatment.

Abelmoschus esculentus (Ladies finger, okra, or gumbo) is a member of the Malvaceae family found across various regions of the world including Africa, Asia, Southern Europe, and America. It is a versatile herb that serves both medicinal and culinary purposes and is a rich source of nutrients. Its consumption has been linked to various health benefits due to its high nutritional content [2].

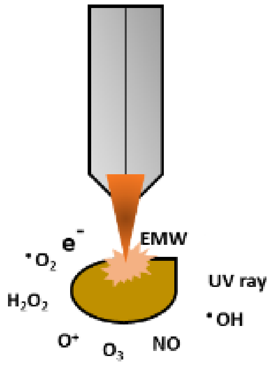

Fig. 1: Illustrates the possible series of events that occur during seed germination initiated by plasma seed priming, according to reference [1].

1.2 Physical Characteristics

Lady's finger is an annual herb and often grows to around 2 meters tall. The leaves are long petioles, orbicular around 10 - 20 cm long, broad and rough, palmately lobed with 5 - 7 lobes, flowers size from 4 to 8 cm in diameter. Fruits are elongated in range 10 to 25 cm in length, 1.5 to 3 cm in diameter. The fruits of Lady's finger plant reach maturity 60 to 180 days after seeding, depending on the cultivar. The plant needs soil that is both moist and well-drained [3]. The current state of the plasma treatment technique's application in plants to study the physical, chemical, and molecular mechanisms underlying different effects [4].

1.3 Chemical Composition

Lady's finger or okra contain fiber up to 67.5 percentage a-cellulose, 15.4% hemi-cellulose, 7.1% lignin, 3.4 percent pectic matter, 3.9 percentage fatty and wax matter, and 2.7 percent aqueous extract on average, according to estimates [5]. Lady's finger is rich source of protein and minerals, including calcium, potassium, iron, and zinc. Gamma-tocopherols, liposoluble pigments, linoleic and palmitic acid are also present in large concentrations in the seeds of all genotypes. Lady's finger is also crucial part of the human diet as it supplies carbohydrates, minerals, and vitamins. Another excellent source of amino acids, fats, fibre, and glucose is the Lady's finger seed. The Fourier transform-infrared spectroscopy (FTIR) of the powder of Lady's finger exhibited functional groups such as CH_3, CH_2, C=C, HC=CH for cabinet-dried powder and CH_3, C-O, COH, HC=CH for solar-dried powder **[6]**. This information can be useful in determining the chemical characteristics and properties of Lady's finger and its potential applications in various fields.

Okra plants have several edible elements that can be used in cooking, including fresh leaves, buds, flowers, pods, stems, and seeds. Okra is consumed as a vegetable and can be used in a variety of dishes such as salads, curries, soups, and stews. Various plasma sources, including DBD jet, microwave discharge, radio-frequency (RF) discharge, and gliding discharge, have been developed and used to treat crops like rapeseed, cotton, maize, oat, wheat, mustard, soybean, legumes, and honey clover as well as vegetables like tomato, radish, coriander, green peas, and sunflower over the past 20 years [7]. Furthermore, it has been discovered that plasma therapy increases overall germination rate, germinating speed. Plasma seed priming has been shown to improve the germination percentage of many crops, including okra.

A cross-disciplinary approach has been gaining considerable interest, and physicists have been actively exploring low-pressure plasma in agriculture. Plasma, an ionized gas produced at room temperature under atmospheric pressure, generates reactive species that can activate plant vitality and inactivate microorganisms, making it a promising tool for increasing crop plant vitality and production [8]. The ability of plasma to enhance seedling growth, plant development and reproduction, and crop sustainability has been the subject of numerous studies. To break dormancy and trigger germination in latent seeds, a number of seed treatment techniques, including scarification, stratification, and chemical methods, were utilized [9, 10].

Some studies related to Lady's figure by different authors are Capsicum annuum [11], Wheat [12] at normal pressure, Raphanus sativus L [13], Glycine max L. Merrill [14], Wheat [15] at low pressure, Cucurbita pepo L. cv.; Cinderella Cucurbita maxima L. cv.; Jarrahdale Cucurbita maxima L. cv.; and Warty Goblin [16], Cichorium intybus [17], Catharanthus roseus [18] and Vitis vinifera [19] seed are treated with DBD plasma in Argon/Air/N_2/He/O_2 environment for enhancing the germination and growth. Astragalus fridae [20], Catharanthus roseus [18], Andrographis paniculate [21], Panax ginseng [22] and Helianthus annus L. (Sunflower) [23] seeds are treated with DBD plasma in Argon environment to study Biophysical and metabolism of DBD plasma-treated seed.

The plasma is a modern technique that promises in mitigating risks associated with agriculture and food processing systems in eco-friendly environments but traditional agrochemical and biotechnological has negative impacts on the environment. The effects of cold atmospheric pressure plasma treatment on the growth of seedlings and germination of seeds under dryness, salt, and chemicals toxicity conditions are being researched more and more [23]. Plasma therapy has been shown in numerous trials to increase resistance to abiotic stress.

In addition, atmospheric pressure plasma has been shown to reduce the water contact angle of seeds, increasing their hydrophilicity and facilitating the uptake of water, which in turn initiates biochemical processes [24]. For instance, Ling et al. [25] study the effect of contact angle on soybean seeds treatment from 70.14^o to 20.94^o. Meng et al. [12] found increased germination rates of wheat seeds treated with DBD plasma in different environment air, nitrogen, and argon, with rises of 24 percent, 28 percent, and 35.5 percent, respectively. The result shows the growth of wheat seeds exposed 10, 20, or 40 minutes, which produced less sprout biomass than seeds treated for 3 minutes. Sarinont et al. [13] discovered that radish seeds allowed to treat with DBD plasma that use air, oxygen (O_2), nitric oxide (NO) (10%), and nitrogen (N_2) environment and found grew of the seedling is more successful than seeds helium (He) and argon (Ar).

2. MATERIALS AND METHODS

For the creation of a seed's measuring system, processing, and storage system, physical qualities are essential for the life cycle of the plant. In this work, a test was done to determine the Lady's finger seed physical and chemical characteristics. The physical characterization of Lady's finger seed used in this work are length, width, thickness, and geometric diameter considered as 4.98 mm, 3.20 mm, 3.39 mm, and 3.98 mm, respectively. The seed size was measured using a screw gauge. The weight on average was around 54.1 g.

2.1 Experimental arrangement

The Plasma Treatment Process involves the use of partially or fully ionized gas that can be ignited at atmospheric conditions and contain charged species such as electrons and negative and positive ions, neutral species such as atomic and/or molecular radicals and non-radicals, as well as electric fields and photons [4]. Over time, various kinds of plasma devices have been developed for studying the physical, chemical, and biological mechanisms of plasma triggered by analysis of its components. Plasma treatment of seeds can be classified into two methods: direct and indirect, depending on the contact of the plasma with the samples. To conduct plasma treatment, plasma sources such as dielectric barrier discharge (DBD), radio frequency (RF) plasma, and atmospheric pressure plasma jet (APPJ) have been used [26]. In this particular work, the authors employed the direct method and used DBD plasma in an Argon environment to treat ladies finger seeds. The experimental setup and treatment process are depicted in Fig. 2.

In order to treat the Lady's finger seeds with plasma, the process shown in Fig. 2 was employed. The plasma was generated in an argon environment at 7kV with a power dissipation of 2.86 W under atmospheric conditions to treat the seed of Lady's finger. Five samples of Lady's finger seeds were taken and each sample was treated continuously for 0.5, 1, 2, 3, and 4 minutes. The treated seeds of 0.5 and 1 minute were germinated to study the physical growth of different parts such as roots, shoots, and leaves.

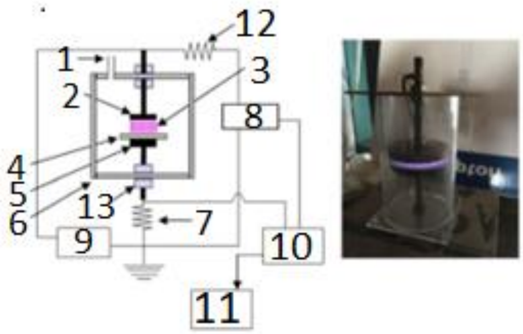

Fig. 2: Schematic diagram of Lady finger seed treatment with DBD plasma: 1. Gasinlet, 2. Parallel disc electrode, 3. Plasma, 4. Glass plate, 5. Grounded discelectrode, 6. Quartz glass chamber, 7. Resistance 10 k:, 8. Voltage probe, 9. AC power supply, 10. Oscilloscope, 11. Computer, 12. Resistance 10 M:,13. Teflon screw

The process of treating seeds through the manipulation of operating parameters, including electrode structure, power source (voltage, frequency, and waveform), discharge gas (air, Ar, He, etc.), and other conditions (gas flow, gas pressure, gas temperature, etc.) is depicted in Fig. 2. In the case of direct plasma treatment, the plasma devices generally comprise a container module with a space to place the seeds, which are then exposed to the plasma generated by the generator and electrode. Surface DBD (SDBD), one of the plasma technologies for biomedical activities, has been more frequently because of its comparatively wider surface area. As shown in Fig. 2, energetic particles, reactive species (including such Hydroxyl radical, singlet oxygen, ozone, and hydrogen peroxide), electromagnetic force, and photons (visible/ultraviolet (UV) radiation) have an immediate effect on the seeds exposed in the discharge area. The combination of these components is considered to be the primary factor that facilitates seed germination and growth. During exposure, the seed surface interacts with short-lived and long-lived radicals that arise due to secondary reactions.

2.2 Impacts of treatment

The physical and chemical alterations to the seed coat or surface are the most commonly reported factors for enhanced seed germination by plasma, as extensively studied. These modifications result in the increased hydrophilicity and water permeability of the seed surface, which aids in water absorption required for seed germination. These effects have been frequently observed in many studies [27]. In addition, the seed surface membrane has been analyzed in several studies for chemical changes and leaching. Moreover, plasma-generated reactive oxygen and nitrogen species (RONS) activate the biochemical and molecular processes inside the seed, which leads to the stimulation of seed germination [28]. The type of RONS produced by the plasma is influenced by the feeder gas. Reactive oxygen species (ROS) facilitate the oxidation of the aleurone layer and mobilize food reserves during seed germination. Hydrogen peroxide (H_2O_2) has been identified as a signaling molecule that stimulates seed germination, with high concentrations observed in cold plasma (Ar/Air, at low pressure) treated seeds. Nitric oxide (NO) is another reactive species that regulates seed germination. In plants, NO is produced from the reduction of nitrate (NO_3^-) to nitrite (NO_2^-) by nitrate reductase, and hence NO_3^- in plasma-activated water is primarily responsible for enhanced seed germination [4, 29]. The addition of RONS species by cold plasma treatment on seed causes the change in the growth rate of the different parts of plants.

3. RESULTS AND DISCUSSION

3.1 FTIR analysis of Lady's Finger

In Fig. 3, the FTIR analysis of untreated and cold plasma-treated Lady's finger seeds in an argon environment at 2.86 W dissipative power is shown. The transmittance of the seeds was observed with respect to the wavenumber, and it was found that the transmittance increases with the wavenumber having an absorption peak. The absorption peak is a result of the stretching of functional groups such as C-H, C=O, N-O, and C-O bonds at different wavenumbers. Table 1 shows the identification of functional groups based on the absorbance peak with respect to the wavenumber. It was observed that the transmittance of seeds treated for 0.5 minutes was higher than those treated for longer durations, indicating that the stretching bond of the functional group is more effective when the seed is treated for 0.5 minutes. This suggests that the plasma treatment affects the functional groups of the seeds, which can have an impact on their growth and development. The transmittance above 1 minute's treatment is found higher than 100% in higher wavenumber regions as shown in Fig. 3.

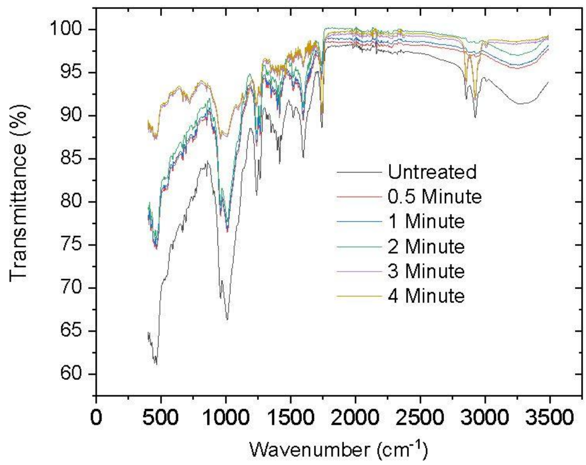

Fig. 3: FTIR analysis of plasma treated Lady's finger seed.

Table 1: Comparison and identification of functional group present on Lady's finger seed

Reference work Absorption Peak (Wavenumber, cm^{-1})	Present Work (Absorption peak, wavenumber cm^{-1})	Functional Group	Reference
2954.00-2918.20	2916.36-2929.87	C-H	[30]
1745.00-1743.00	1735.93-1743.64	C=O	[31]
1600.00-1500.00	1593.20-1597.05	C=O	[32]
1400.00–1290.00	1415.75-1234.44	N–O	[33]
1000.00-1074.00	1006.84-1010.70	C-O	[34]

Observations were made on the transmittance of Lady's finger seeds treated with cold plasma for 0.5 to 4 minutes, and compared with untreated seeds. It was found that the transmittance of the treated seeds was higher than that of the untreated seeds. This increase in transmittance is attributed to the bond stretching of the functional groups present in the seeds. The change in bond length varies with the function of the group, causing the variation in transmittance. Further analysis of the transmittance of IR through different functional groups revealed that the transmittance of IR through the C-H bond of the untreated seed was less than 1.30% of 0.5 minutes treated and 8.80% of 1 minute treated. The transmittance of IR through the C=O bond (1735.93-1743.64) of the untreated seed was greater than 0.24% of 0.5 minutes treated and less than 5.83% of 1 minute treated. The transmittance of IR through the C=O bond (1593.20-1597.05) of the untreated seed was less than 5.51% of 0.5 minutes treated and greater than 4.91% of 1 minute treated. The transmittance of IR through the N-O bond of the untreated seed was less than 6.26% of 0.5 minutes treated and greater than 4.67% of 1 minute treated. The transmittance of IR through the C-O bond of the untreated seed was less than 27.20% of 0.5 minutes treated and 14.08% of 1 minute treated. These observations suggest that the bond length increases with cold plasma treatment, resulting in changes in transmittance as shown in Fig. 3. These changes in bond length have a significant effect on the growth of Lady's finger plants, as shown in Fig. 4. The transmittance of treated seed above 1 minute is found higher than other (untreated 0.5 and 1 minutes). This is because of function group bond strength due to energy gain during treatment. The identification of the function group is shown in Table 2 up to 1 minute treatment and the same corresponding group above 1 minute treatment.

Table 2: Comparison of transmittance at absorbance peak at different treated time

Present Work (Absorption peak, wavenumber cm^{-1})	Functional Group	Transmittance		
		Untreated treated	0.5 minute treated	1 minute treated
2916.36-2929.87	C-H	90.51	91.74	96.82
1735.93-1743.64	C=O	89.08	88.87	94.21
1593.20-1597.05	C=O	85.42	90.03	89.49
1415.75-1234.44	N–O	85.51	91.04	89.60
1006.84-1010.70	C-O	66.58	87.54	76.67

3.2 Growth of root, shoot and leaves of plasma-treated Lady's finger seeds after germinated

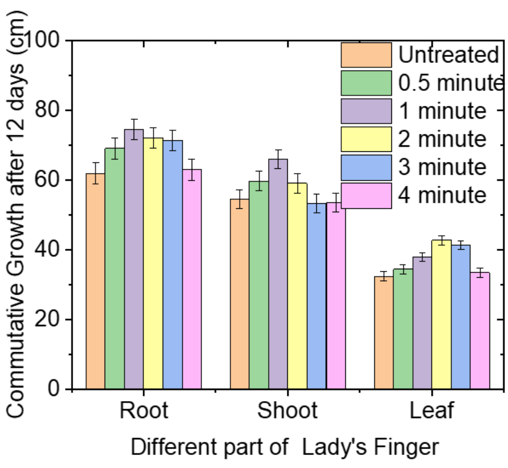

Fig. 4: Growth of Root, Shoot and Leaves of Lady's Fingers.

In Fig. 4, the growth of the Lady's finger's different parts, including the root, shoot, and leaves was studied after the germination of the seed. The measurement of different parts Lady's finger was considered after 12 days of germination for this research. A similar measurement was also done after 24 days of germination as shown in Fig. 5. The results show that the growth of several plant components was influenced favorably by Lady's finger seeds that had undergone plasma treatment. When plasma was applied to sprouted Lady's finger seeds for 0.5 or 1 minute, it was discovered that the root growth rate was 10.84% or 18.38% higher than that of untreated seeds. The shoot growth rate of the germinated Lady's finger seeds treated with plasma for 0.5 minutes was 9.54% greater, while for those treated for 1 minute, the rate was 21.10% greater than untreated seeds. The leaf growth rate of the germinated Lady's finger seeds treated with plasma for 0.5 minutes was found to 3%, while for those treated for 1 minute, the rate was 15.64% higher than untreated seeds. Additionally, Ling et al.'s study on the seedling growth of plasma-treated soybean seeds found that the shoot and root growth rates increased by 13.77% and 27.51%, respectively [27]. These findings highlight the positive effects of plasma treatment on seedling growth and development, particularly in terms of root and shoot growth. Further studies could investigate the optimal plasma treatment conditions for maximizing the benefits on plant growth while minimizing any potential negative effects. Fig. 4 also represents that the growth of the root is higher when the seed is treated for 3 minutes while the shoot is higher when the seed is treated for 1 minute but the leaf is higher when the seed is treated for 2 minutes. This shows the plasma has an impact on the physical growth of Lady's growth but is not uniform.

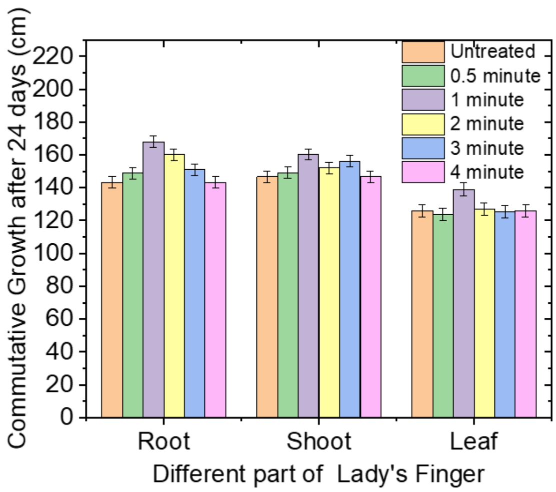

Fig. 5: Growth of Root, Shoot and leaves of Lady's Finger.

It has been determined that the generation of reactive nitrogen and oxygen species has the greatest impact on how seeds respond to plasma treatment (RONS). The generation of RONS from plasma has been identified as a crucial factor in promoting seed germination, seedling growth, and plant defense mechanisms. When the root, shoots, and leaves of the Lady's finger plant were observed. The observation shows that the shoot and leaves growth decreased when the measurement was taken after 24 days of germination for the 0.5 minute treated time of seed. In addition, observation shows that the shoot and leaves growth increased when the measurement was taken after 12 days (earlier days) of germination for the same treated time (0.5 minute). Fig. 6 further demonstrates that while the rate of growth of the roots was uniform, that of the shoots and leaf was not. This demonstrates that RONS have variable effects on plant growth depending on their quantity and exposure duration. Low levels of RONS can promote the growth and development of plants, whilst large levels can have the opposite effect. Overall, the findings imply that treatment can have a major impact on plant growth and maturation, with the effects perhaps varying with treatment time and intensity. Further research could explore the optimal conditions for plasma treatment to maximize the benefits and minimize any potential negative effects on plant growth.

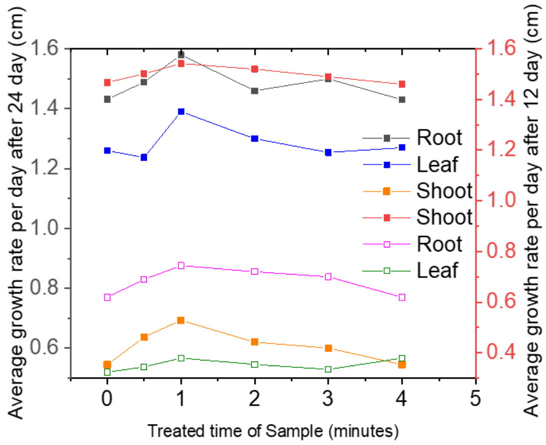

Fig. 6: Average grow rate of different part of Lady's finger.

From fig. 4, fig.5 and fig. 6, it is observed plasma has effect on seed germination and the effect of plasma at different part of Lady's finger is different.

3.3 Chemical and Physical Effects of Plasma

The production of reactive oxygen and nitrogen species (RONS) from plasma is considered a crucial factor in enhancing seed germination, seedling growth, and plant defense mechanisms. Mechanical changes to the seed's surface or coat are what are referred to as plasma-induced seed germination; however, additional factors like heat, seed sterilization, Ultraviolet rays, ionizing, and electromagnetic fields produced by plasma devices also play a role. We have outlined the key elements so that you can better understand how plants are treated with plasma. Reactive oxygen species (ROS) and reactive nitrogen species (RNS), which comprise ozone, hydrogen peroxide, superoxide anion, peroxyl, nitric oxide, nitrogen dioxide, and peroxynitrite, are two categories for the reactive species produced by plasma therapy. As seen in Fig. 7, studies have revealed that all these reactive species play a significant role in influencing seedling growth and plant growth at various stages. The treatment process has been proven to have a good impact on plant growth and maturation by altering the molecule of the seed and encouraging nutrient absorption and cell division. Overall, treatment has shown to be a promising method for enhancing plant growth and seed germination. The processes behind the benefits of plasma therapy might be explored in more detail, and treatment conditions could be improved for the greatest possible advantages.

Table 3: Growth rate of different part of the lady's finger plant

Parts of Lady's finger	Growth rate with Untreated (cm per day)	Growth rate with 0.5 minute (cm per day)	Growth rate with 1.0 minute (cm per day)
Root	0.56	0.58	0.63
Shoot	0.45	0.48	0.58
Leaf	0.28	0.30	0.33

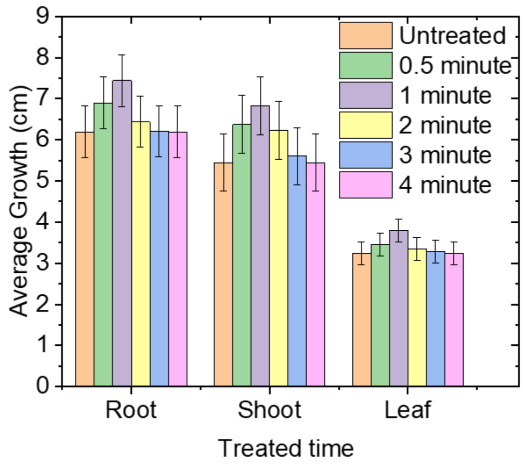

Fig. 7: Comparison of the growth of different part of Lady's finger parts with error bar.

In Fig. 7, a comparison of the physical growth of the Lady' finger plant, specifically the root, shoot, and leaf, is presented. The results indicate that the growth of the root, shoot, and leaf of the Lady's finger plant increased with the duration of cold plasma treatment. However, it was observed that the growth rate of the leaf was lower compared to that of the shoot and root. This difference in growth rate could be attributed to the fact that the cold plasma treatment primarily affects the cellular structure and function of the plant, which can lead to changes in the physical growth of the plant. The observed increase in the growth rate of the shoot and root could be due to improved nutrient uptake and increased cell division and elongation, while the lower growth rate of the leaf could be due to changes in the leaf structure or function. Overall, the results suggest that cold plasma treatment has the potential to positively impact the physical growth of plants, such as the Lady's finger plant. Further studies could explore the underlying mechanisms of these effects and optimize the cold plasma treatment conditions to maximize the benefits for plant growth and development.

4. CONCLUSION

The treated seed of Lady's finger was analyzed using Fourier Transform Infrared (FTIR) spectroscopy analysis technique to identify the functional groups. The FTIR analysis technique of functional groups shows the presence of C-H, C=O, C-O, and N-O. The study of transmittance of treated seed shows transmittance varies with plasma treatment time. Also, the study shows the bond length of the functional groups of treated Lady's finger seeds was found greater than untreated. Furthermore, the physical growth of the treated seeds was compared to the untreated seeds after germination. The results showed the growth rate of the roots, shoots, and leaves is higher than untreated. This indicates that cold plasma has a positive effect on the physical and chemical properties of Lady's finger seed.

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