EFFECT OF GLIDING ARC DISCHARGE PLASMA ON GERMINATION AND PRODUCTION OF OYSTER MUSHROOM (*Pleurotus ostreatus*)

A Dissertation

Submitted to the Dean Office, Institute of Science & Technology, Tribhuvan University, Kirtipur in the Partial Fulfillment for the Requirement of Master's Degree of Science in Physics

By

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December, 2022

RECOMMENDATION

This is certified that **Mr. Deepak Niure** has carried out the dissertation entitled **"EFFECT OF GLIDING ARC DISCHRAGE PLASMA ON GERMINATION AND PRODUCTION OF OYSTER MUSHROOM (*Pleurotus ostreatus*)"** under my supervision.

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EVALUATION

We certify that we have read this dissertation and in our opinion it is good in the scope and quality as dissertation in partial fulfillment for the requirement of Master's Degree of Science in Physics.

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Abbreviation

ANOVA: Analysis of variance

BE: Biological efficiency

GAD: Gliding arc discharge

LSD: Least significant difference

LMS_1: 1 min seed treated by plasma

LMS_2: 2 min seed treated by plasma

LMS_3: 3 min seed treated by plasma

$LPAW_1$: 4 min treated water

$LPAW_2$: 8 min treated water

$LPAW_3$: 12 min treated water

LST_1: 5 min substrate treated by plasma

LST_2: 10 min substrate treated by plasma

LST_3: 15 min substrate treated by plasma

LT_0: Control (untreated sample)

NARC: National Agriculture research council

NIST: National Institute of Standards and Technology

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ABSTRACT

An experiment was conducted at plasma lab of the Central Department of Physics, Tribhuvan University, Kirtipur Kathmandu Nepal to study the effect of cold plasma on the germination and production of oyster mushrooms (*Pleurotus ostreatus*) by gliding arc discharge. The treatment includes four different methods for 10 samples on two replicate. Control, spawn treated with plasma, plasma activated water used for spraying and substrates treated with plasma. The main parameters measured during the experiment were the colonization and fruit appearance periods, the length and diameter of the stem and cap (pileus) diameters; the fresh weight of the first and second harvests of mushrooms, and the biological efficiency of various treatment methods. Among the different treatments of plasma, the time for colonization and fruit appearance was found to be faster than control. The length of stem was highest for spawn treated with plasma for three minutes, but the diameter of the stem was found to be the highest for treatment four minutes plasma treated water used and the diameter of cap found most significantly highest for spawn treated with plasma for three minutes. Similarly, the production and biological efficiency were found to be significantly highest in the case of spawn treated with plasma for two minutes, followed by substrates treated for ten minutes with plasma , and eight minutes of plasma activated water used respectively. Plasma treatment on oyster mushroom can play a significant role in the growth and production of mushrooms.

Key Words: gliding arc plasma, plasma activated water (PAW), mushroom, cap, stem, production

CONTENTS

CHAPTER 1

INTRODUCTION AND BACKGROUND

Chapter 1

Background / Introduction

In this revolutionary period of science and technology, plasma technologies influenced the agricultural industry along with other various applications of it. Numerous researchers are working and studying the effect of plasma treatment on crops, seeds, and soil. In the study of plasma treatment, plasma contributes to the seed germination, seed disinfection, and inactivation of microorganisms on food, and insect control, which altogether can contribute to an increase in crops production [1]. Mushrooms are highly nutritious and environmentally friendly crops that carry numerous medicinal benefits. The cultivation of edible mushrooms carries great relevance in today's world in the context of burgeoning population growth and extreme pressure on the environment. But advances in research on mushroom breeding and production are very limited as compared to other crops. This may be partly due to a lack of previous knowledge of the genetics and breeding system in this crop [2]. But still, to date, there is not much study and research on plasma's effect on mushroom germination and production. Therefore we have a curiosity to understand the effect it shows on the mycelium germination duration, length of the stipe, cap diameter, and production. By the method of gliding arc discharge. Context of Nepal, Nepal is an agricultural country having 66 percent of people directly involved in farming. Most Nepalese directly grow crops without using any scientific tools in farming. Livestock is one of the important sources of cash income for farm households.

1.1 Plasma

Plasma is a quasi-neutral gas of charged and neutral particles that exhibit collective behavior. In terms of energy, Plasma is the fourth state of matter, apart from every solid, liquid, and gaseous states as illustrated in Figure 1.1. In physics, plasma is an "ionized" gas in which at least one of an atom's electrons has been stripped away, leaving a positively charged nucleus, called an ion. Plasma is

sometimes called the "fourth state of matter". When a solid is heated, it becomes a liquid. Heating a liquid turns it into a gas. On further heating, the gas is ionized into plasma. Since plasma is made up of charged ions and electrons, electric fields run rampant everywhere, and particles "clash" not only when they collide, but even at a distance where they can sense electricity [3].

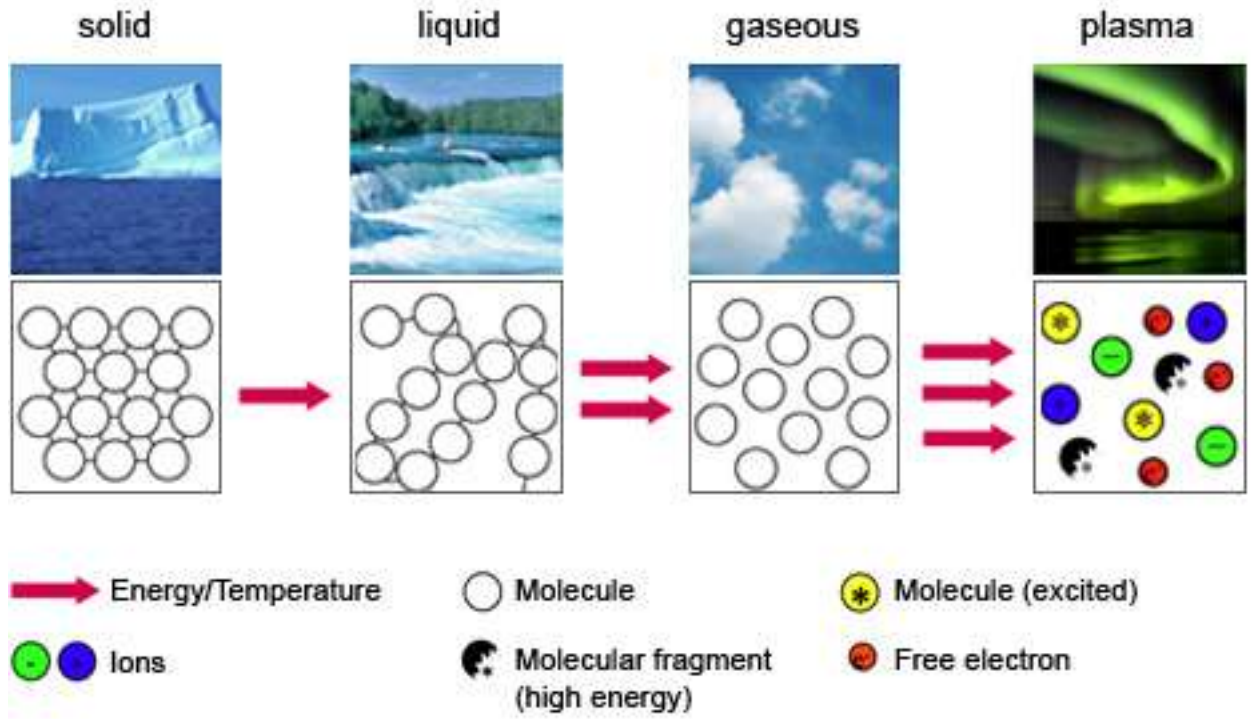


Figure 1.1: Plasma-fourth state of matter [4].

Plasmas have features that scale across several orders of magnitude and differ in terms of temperature and density. Depending on its nature, plasma has a variety of applications. There are two main forms of plasma:

(a) Thermal Plasma

(b) Non-Thermal Plasma

Hot plasmas can occur in one of two situations: when the pressure is atmospheric, even at temperatures as low as 6000K, or when the heavy particles are extremely energetic, at temperatures on the order of 10^6–10^8 K(10^2–10^4 eV) [5]. The study of thermal plasma has advanced significantly in torch design, understanding of coupling (RF) and electrode phenomena, calculation of thermodynamic and transport properties, modeling of plasma flows with or without current carrying, and measurement of plasma temperatures, velocity, composition, and parameters (velocity, trajectory, surface temperature) of the particles in flight within the plasma jets [6].

When the temperature of the electrons is substantially greater than that of the heavy particles—electron temperatures can reach values of 10^4–10^5 K(1–10 eV) or when the temperature of the gas is as low as

ambient temperature, cold plasmas can also occur. Cold plasma is a kind of plasma with an uneven distribution of energy, or "none equilibrium." Among the many uses for plasma, cold plasma (non-thermal plasma) is also particularly beneficial in fields including surface modification, the production of micro-and integrated circuits (IC), metallurgical applications, fabric treatment, and agriculture [5].

Application Of Plasma

Although plasma has several uses in this context, we are primarily interested in non-thermal plasma. The primary application of non-thermal plasma in NTP is decontamination in food processing environments, which includes the dry disinfection of food surfaces (meat, poultry, fish, and recently harvested horticultural produce), granular and particulate foods (dried milk, herbs, and spices), and sprouted seeds. After the banning of ethylene oxide gases, there is a significant opportunity for NTP sterilization of particulate foods. The surface sterilization of packing materials using this approach was successful. Research on cold plasma is now concentrated on its uses for food decontamination, toxin elimination, and packaging improvements. A variety of enzymes have reportedly also been inactivated by it [7-9].
Some of the applications of non-thermal plasmas in agriculture are :

- Decontamination of Seeds
- Enhancement of Seed Germination
- Growth of Plants
- Soil Remediation

Cold plasma processing systems have several benefits in agriculture. They also do not harm crops, foods, seeds, people, or the environment. Reactive neutral species, charged species (electron, ion), electric fields, and UV light are all produced during plasma discharges. These variables influence seed germination, plant development, and the quality of agricultural products through changing the density of reactive oxygen species (ROS), reactive nitrogen species (RNS), pH, oxidation-reduction potential, electrical conductivity, and other variables [1].

1.2 Mushroom

The fleshy, spore-bearing fruiting body of a fungus that grows above ground, on soil, or on its food supply is known as a mushroom [10]. Mushrooms are consumed both as food and medicine due to their

great nutritional content. Mushrooms typically have a 19–35% protein content based on dry weight. All of the necessary amino acids are present in the mushrooms' proteins, which are also particularly high in lysine and leucine, two amino acids that are typically absent from diets. Fresh mushrooms have comparatively high amounts of carbohydrate and fibre due to their low total fat content and high proportion of polyunsaturated fatty acids in relation to the overall fatty acids. Mushrooms seem to be high in vitamins and minerals such as thiamine, riboflavin, niacin, biotin, and ascorbic acid [11].

Figure 1.2: Oyster mushroom [12].

There are many different kinds of mushrooms; some are edible and some are not. Globally, only edible mushrooms are grown on the farm. Due to the limited resources and lack of scientific understanding in a nation like Nepal, the traditional method of mushroom production is employed. The Nepal Agricultural Research Council (NARC), Division of Plant Pathology, began cultivating mushrooms in 1974. Utilizing the synthetic media of paddy straw, the growing method for white button mushrooms was created in that early period and made available to all farmers starting in 1977. Since 1984, when farmers were first given the technique to cultivate oyster mushrooms using packets of chopped straw, mushroom cultivation has gained popularity [13]. Agaricus bisporus (Gobre Chyau), Pleurotus ostreatus(Kanne Chyau shown in Figure 1.2), Laccaria leccata (wood mushroom), oyster, volvarilla, shitake, ganodirma, etc. are some of the mushroom species that are often cultivated in Nepal [14].

Due to the lack of proper methodology and scientific tools, many farmers are not doing so well in farming for the crops they grow. We need to implement the proper scientific methods of agriculture. In this study, we are using atmospheric plasma to implement plasma physics for mushroom growth and production.

CHAPTER 2

LITERATURE REVIEW

Chapter 2

LITERATURE REVIEW

Mishra et al. studied on non-thermal plasma inactivation of food-borne pathogens. Studies have shown that NTP can be used for the surface decontamination of raw produce, including dried nuts and packing materials. They also review the actions of plasma agents on the microbial classes and describe applications that have been successfully used and those that have the potential to be used in food processing [7]. Sharma et al. studied about *Pleurotus ostreatus* was grown on a variety of substrates, including rice straw, rice straw with wheat straw, rice straw with paper, sugarcane bagasse, and alder sawdust. 10% more rice bran was added to each substrate, except for the rice straw. Regarding mycelial growth, colonization time, primordial appearance time, mushroom yield, biological efficiency (BE), mushroom size, and chemical composition, the impacts of various substrates were examined. The optimum substrate for the growth of mushrooms was determined to be rice straw (control), followed by rice combined with wheat straw and rice straw combined with paper scraps. Mushroom fruit cultivated on rice straw had a greater nutritional content than others [15]. Ling et al. investigated cold plasma treatment's effects on soybean seed germination and seedling development. Seeds were pre-treated with cold plasma for 15 seconds. The results demonstrated that plasma treatments improved seed germination and seedling development. The seed's ability to absorb water improved, the germination and vigor indices were significantly raised, and the apparent contact angle was reduced. Shoot length, shoot dry weight, root length, and root dry weight all increase significantly as seedlings grow. Cold plasma treatment considerably increased the seed reserve utilization, including weight of the mobilized seed reserve, seed reserve depletion percentage, and seed reserve usage efficiency. demonstrating that plasma treatment grew plant roots more effectively [16]. Xu et al. investigated the effect of plasma activated water on the post-harvest quality of button mushrooms using a non-thermal, atmospheric-pressure plasma jet. When kept at 20 °C for seven days, plasma-activated water soaking was used to study the post-harvest preservation of button mushrooms (*Agaricus bisporus*). Plasma activated water

reduced the microbial counts by 1.5 log for bacteria and 0.5 log for fungi, respectively, during storage. According to the data regarding respiration rate, relative electrical conductivity, and hardness, plasma-stimulated water soaking could delay the softening of mushrooms. However, plasma activated water did not appreciably alter the color, pH, or antioxidant properties of A. bisporus. After harvest, soaking in plasma-activated water is one method that can help keep A. bisporus fresh [17]. The effects of cold atmospheric plasma (CAP) treatment on the substrate for mushroom growth were analyzed by N. Redzuan et al. by combining Pugh's approach with the morphology chart. With the chosen treatment time, the substrates were aligned just under the plasma tube with a spacing of 10 mm. The samples treated for 5, 10, and 15 minutes at 5 kV supplied voltage were contrasted with an untreated sample used as a control. Colony Forming Unit (CFU) testing was used to analyze the quantity of bacterial growth on treated and untreated mushroom substrates, and it was discovered that all CAP-treated substrates had significantly less bacterial growth [18]. Agun et al. studied the impact of cold plasma on the germination and growth of oyster mushrooms. The mushroom spawn grains were produced toward the spawn by utilizing a cutting-edge atmospheric cold plasma pen system. By considering different plasma exposure times (0, 5, 15, 30, 45, and 60 seconds), at atmospheric pressure, with flow rates of 4, 5, and 6 SLM, and a supply voltage of 7 kV The findings demonstrate that the flow rate, cold plasma processing parameters, and treatment duration have a significant impact on the germination and growth of mushrooms. When compared to control spawn grains, the CP pen system, which is optimized at 5 SLM and 15 s, triples the weight of the mushrooms it produces and accelerates the rate of mycelium growth (to 4 weeks) compared to control spawn grains(6 weeks) [19]. Agun et al. researched on the sterilization of oyster mushroom crop residue using cold plasma discharge, this method acts as a sterilizer, killing soil-borne pathogens without affecting the substrate qualities. Onto the crop residue substrate, the plasma with a variable sterilizing time was discharged. According to the study, the bacteria colonies decreased when the sterilizing time was increased. Two days earlier, the fruiting body of the mushroom was effectively cultivated on a crop residual substrate that had undergone cold plasma sterilization. The best optimum time, according to the results, is 25 minutes for sterilizing using cold plasma technology. The fruiting body grew more quickly, the sterilizing process took less time, and the number of soil-borne pathogens was decreased. The mushrooms' production also increased [20].

Agun et al. claim that cold plasma efficiently inhibits the growth of bacteria colonies on oyster mushroom surfaces. The mushroom surface was subjected to the cold plasma discharge with a configurable exposure period, 6 kV of power voltage, and 5 ltr/min of atm flow rate due to the design of the dielectric barrier discharge-cold plasma pen (DBD-CPP) system. The screening of the results

reveals that bacterial colonies did not develop at exposure treatment intervals of up to 3 minutes. This is because the plasma bombardment destroyed the bacteria's cell wall. By eliminating the bacteria on the mushroom surface, this study was able to prolong the shelf life of the mushroom and create a fresh mushroom that was free of microbes [21].

2.1 Objectives

The objective of this research is to study the effect of cold atmospheric plasma on Oyster mushroom germination and production. The objectives of this research work are:

- To study the impact of gliding arc discharge plasma on the germination and production of mushrooms.
- To study the impact of plasma on mycelium growth, stem length and diameter and on cap diameter.
- To study the effect of plasma on the total production of mushrooms.

CHAPTER 3

METHODOLOGY AND DATA ANALYSIS

Chapter 3

Methodology

Experimental Sites and Design

The experiment was conducted on the mushroom (*Pleurotus ostreatus*) in the plasma lab (nuclear) of the Central Department of Physics at Tribhuvan University, Kirtipur. The time of experiment conducted was from 23 march 2022 to 08 may 2022. The wheat grain spawn of *Pleurotus ostreatus* was obtained from the NARC. The experiment was carried out by the 10-treatment method.

1. LT_0= Control (untreated sample)
2. LMS_1= 1 min seed treated by plasma
3. LMS_2= 2 min seed treated by plasma
4. LMS_3= 3 min seed treated by plasma
5. $LPAW_1$= 4 min treated water by plasma
6. $LPAW_2$= 8 min treated water by plasma
7. $LPAW_3$= 12 min treated water by plasma
8. LST_1= 5 min substrate treated by plasma
9. LST_2= 10 min substrate treated by plasma
10. LST_3= 15 min substrate treated by plasma

3.1 Plasma Diagnostics

Producing plasma is not sufficient; it must also be characterized before it can be used in the necessary region. For plasma diagnostics, there are several techniques. We have performed electrical and optical characterization. Schematic diagram of plasma characterization is shown in Figure 3.1.

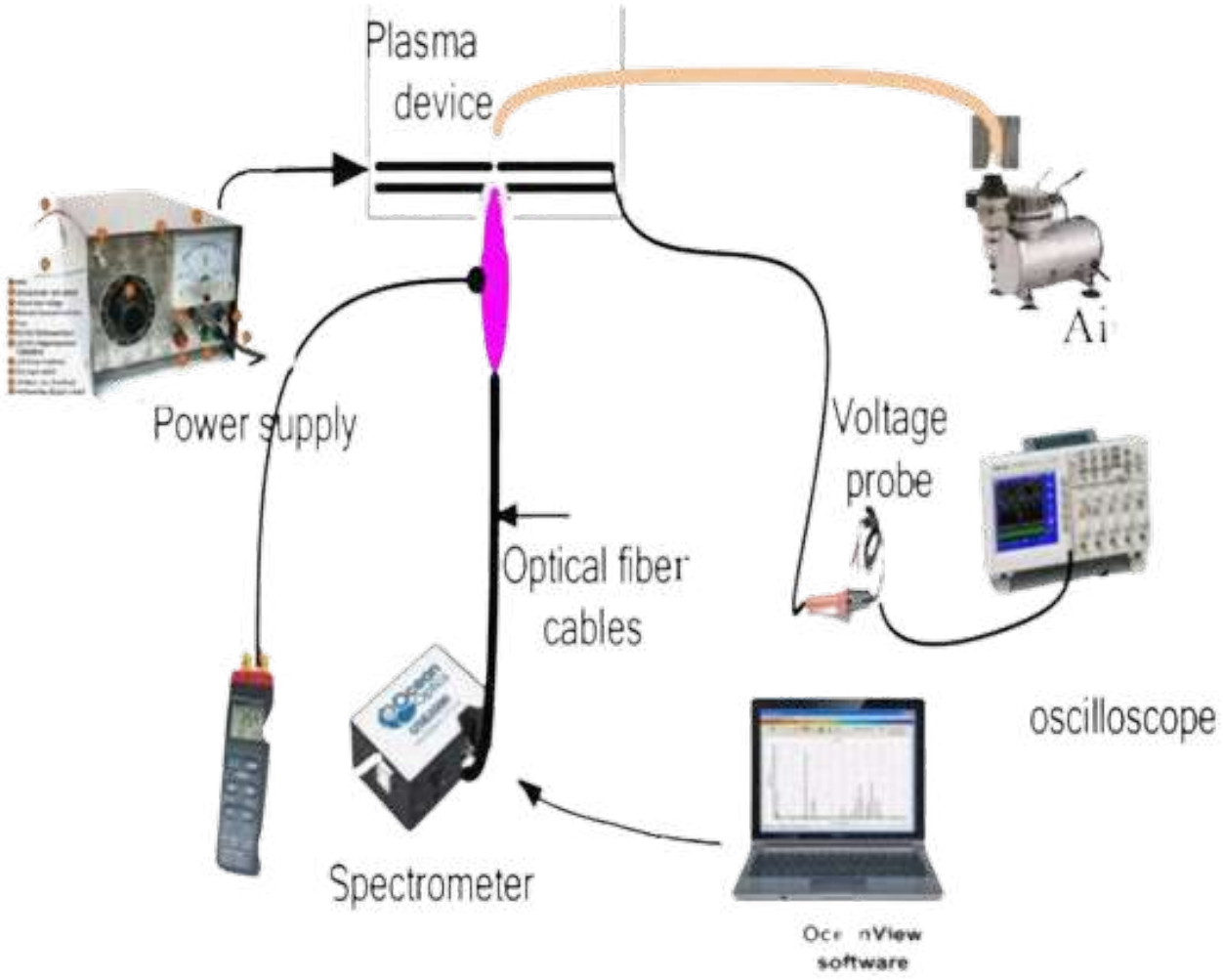


Figure 3.1: Schematic diagram of plasma characterization.

3.1.1 Electrical Characterization

The voltage and current generated during plasma formation will be determined by the electrical characteristics of the plasma. The oscilloscope is linked to one end of the high voltage probe, which is in connection with the equipment on the other end. In this procedure, two channels are used so that voltage and current can be analyzed simultaneously. Voltage and current waveforms are examined using a Tektronix TPS2012B oscilloscope (Figure 3.2). The data from the oscilloscope is plotted

in origin to determine the precise value of voltage and current. A graph showing the peak-to-peak voltage and current values indicates the voltage. However Ohm's law is used to compute current.

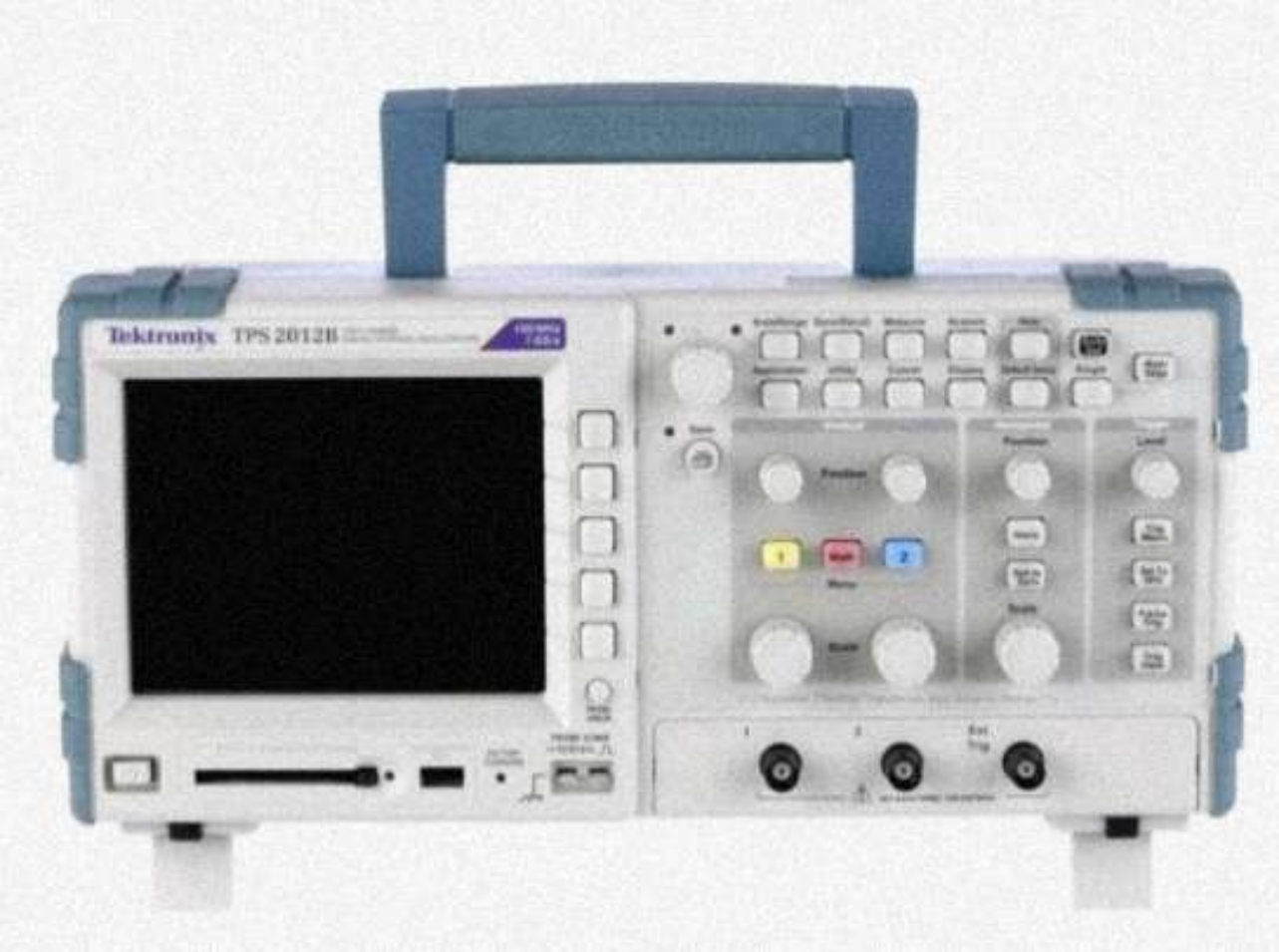


Figure 3.2: Tektronix TPS2012B oscilloscope

3.1.2 Optical Characterization

In this work, the generated plasma is optically characterized using a spectrometer HR4000CG-UV-NIR shown in Figure 3.3, that can identify species with wavelengths between 200 nm and 1.1 m. This spectrometer uses optical fiber to detect the discharge created in the plasma, which is placed about 3 cm away from the discharge produced. To save the information gathered by the spectrometer, the cable was attached to a computer. Later, the data was plotted from the origin software to identify the species of discharge.

Figure 3.3: HR4000CG-UV- NIR Spectrometer

3.2 Gliding Arc Discharge(GAD)

A system in between thermal and non-thermal discharges is the gliding arc (GA). With a high degree of non-equilibrium, high electron temperature, low gas temperature, and the potential to stimulate certain chemical processes without quenching, it may simultaneously offer high plasma density, power, and operating pressure Figure 3.4 shows the Schematic diagram of Gliding arc discharge [22].

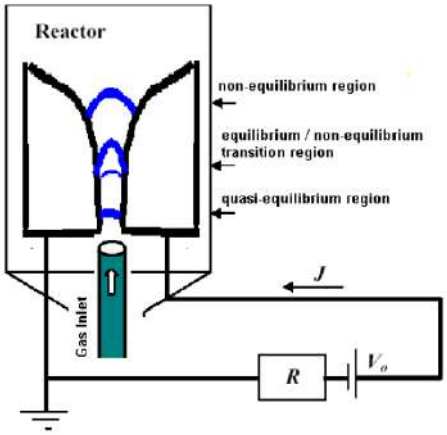


Figure 3.4: Schematic diagram of Gliding arc discharge(GAD).

3.2.1 Experimental setup

The gliding arc experiment consists of two identical electrodes made of copper with an arc length of about 6.6 cm. The gap between the two electrodes is a few millimeters, one of them is connected to the DC power supply (Figure 3.6), and the other one is connected to the ground. The gas was injected from a narrow tube located in a narrow gap between two electrodes.

Figure 3.5: Gas flow meter.

The gas flow can be controlled via flow meter measured (Figure 3.5) in litre/min. The two electrodes and the gas pipe were located inside the plastic cap. The airflow is made constant (12 ltr/min) throughout the whole experiment.

Figure 3.6: Power supply made for plasma production in laboratory of CDP.

3.3 Substrate Preparation and Bagging

The treatments consist of rice straw. The substrates were chopped into small pieces (Figure 3.7) of 1-2 inches, 24 hours soaked, and thoroughly wash with clean water, and excess water will be allowed to drain down so that water didn't ooze out when squeezed with a hand.

Figure 3.7: Preparation of substrate (rice straw) for sterilization.

Then each substrate was individually sterilized by boiling using the metallic pot for 2 to 3 hrs and was allowed to cool to the normal temperature which is then filled into plastic bags making a layer of 50 counting spawn with two layers in each plastic bag.

(a)

(b)

(c)

Figure 3.8: After sterilization and plasma treatment(a) mushroom spawn (b) spawning (c) bagging.

Spawning was done at the periphery of each layer, then after the bags were tied at the mouth and a few holes are made for aeration around the periphery plastic bag and were incubated in the dark and ventilated room at around room temperature by maintaining humidity above 70%.

Plasma treatment on Spawn, Water & Substrate

To treat mushroom spawn with plasma, the mushroom spawn is first numbered and placed inside a plastic bottle that has been split in half. When the plasma is allowed to enter the spawn, the distance between the spawn and the electrode tip is measured, and this distance is 0.4 mm. the mushroom spawn is treated in this manner with various treatment times (1 min, 2 min, and 3 min).

To create each category's plasma-activated water, 200 ml of water is poured into the measuring glass, which is then positioned above the magnetic stirrer and below the electrode of gliding arc. The treatment was supplied at various intervals of time (4 min, 8 min, and 12 min) shown in Figure 3.9.

(a) **(b)** **(c)**

Figure 3.9: Plasma treatment in the laboratory(a) mushroom spawn (b) Water (c) Substrate.

For the plasma treatment on the substrate, the substrate was placed inside the glass block and plasma is allowed to pass into the block for various intervals of time (5 min, 10 min, and 15 min).

To measure the total dissolved solids (TDS), oxidation-reduction potential (ORP), pH value, and electrical conductivity (EC) of the tap water collected in the tank above the Central Department of Physics (CDP), we use a multiprobe pH meter (Figure 3.10).

Figure 3.10: Multiprobe pH meter.

Harvesting, Data Collection and Analysis

The mushrooms were harvested with the help of a knife as soon as they attain the proper size. The data regarding the during the period of colonization and fruit initiation, the fresh weight of the mushroom by weighting balance Figure 3.11.

Figure 3.11: Weighting balance

The diameter of the mushrooms harvested during the first and second flushes depends on the cap, stem, and length of stem were taken as shown in Figure 3.12.

Figure 3.12: Measuring the diameter of the stem.

BE was calculated by dividing the fresh weight of harvested mushrooms (g) by the dry weight of Substrate [23]. Finally, the analysis of the data was carried out using LibreOffice Calc, one way ANOVA and SLD test at 5% level of significance.

CHAPTER 4

RESULTS AND DISCUSSION

Chapter 4

Results and Discussion

In the Central Department of Physics lab, gliding arc discharge plasma was produced. The plasma was then characterized using spectroscopic techniques (electrical and optical characterization).

4.1 Electrical Characterization Plasma

Plasma can be electrically characterized to provide voltage and current data. To get current wave forms, high voltage probes are maintained across high resistors to maintain Ohm's law. The oscilloscope's data can be plotted to produce the current and voltage waveforms as shown in the Figure 4.1.

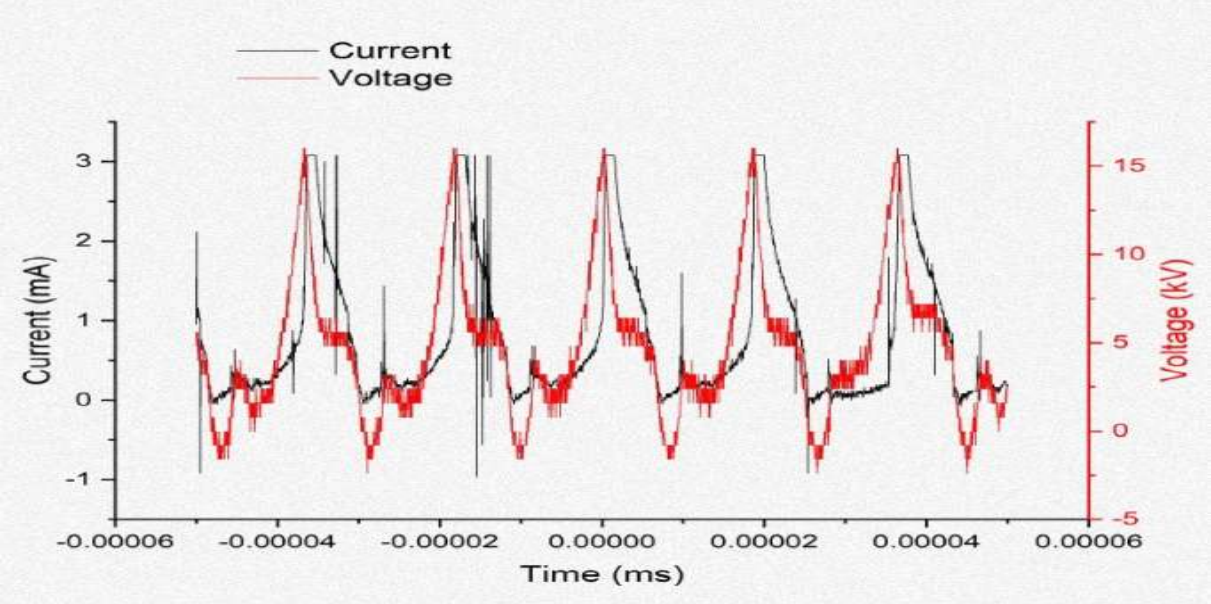


Figure 4.1: Current and voltage graph of gliding arc.

From Figure 4.1, the peak voltage and current for a gliding arc discharge are 17.6 kV and 1.83 mA, respectively.

4.2 Optical Characterization Plasma

Different species found in plasma need to be characterized. Variables like the applied voltage, the electrode being used, the plasma's temperature, the space between the electrodes, and many more affect the species found in plasma. By maintaining a 5 mm gap between the electrodes for GAD, optical characterization of the plasma is accomplished. To determine the wavelength and intensity of the discovered species, data is plotted in origin. We use the NIST database to determine the exact species shown in Table 4.1. The closest wavelength is looked for, and the relevant species is recorded. Keeping all other parameters constant, optical emission spectra were studied by varying the speed of the blower. The data obtained was plotted in origin simultaneously, which is shown in the Figure 4.2.

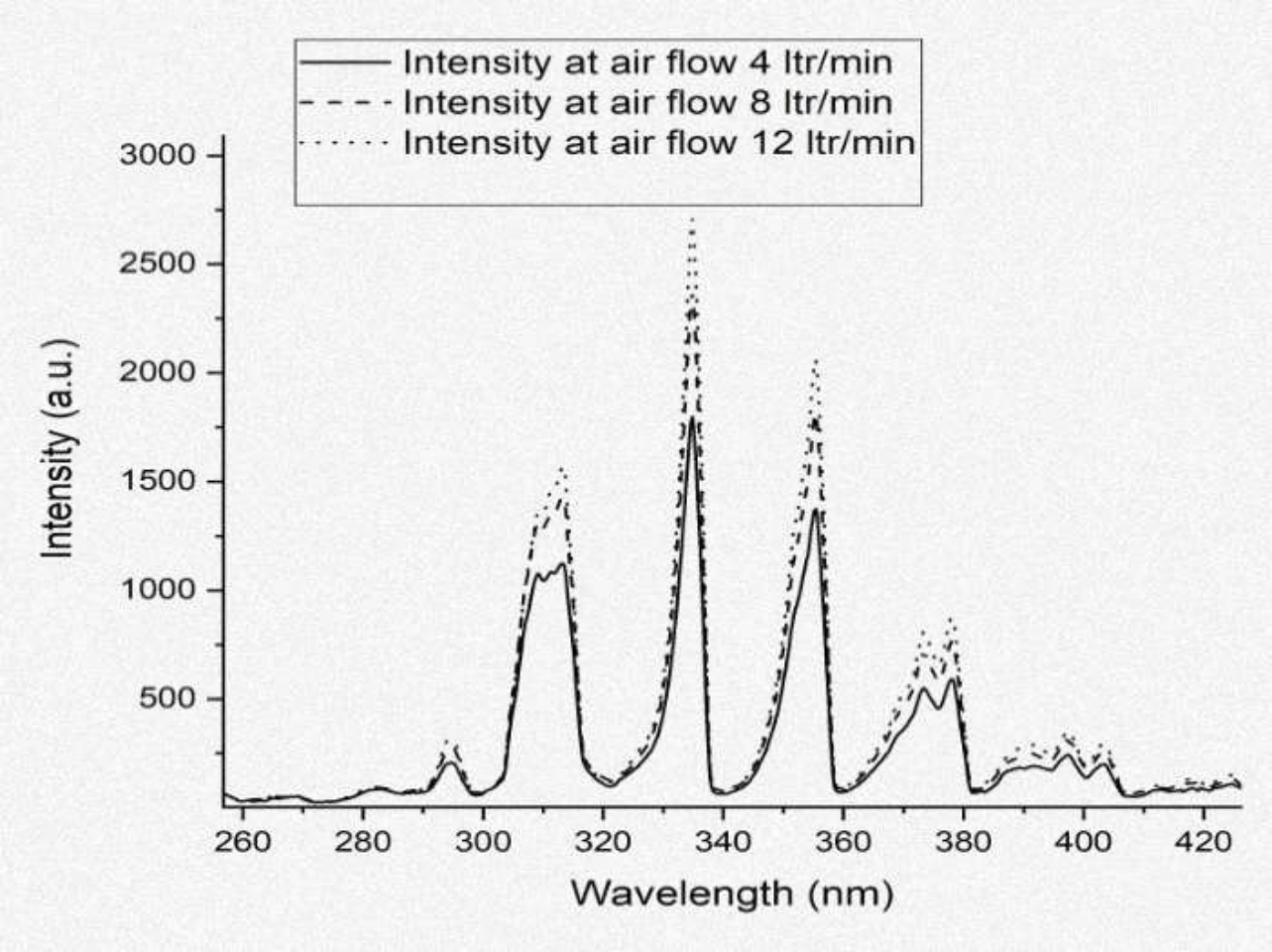


Figure 4.2: Optical emission spectra of gliding arc at different air flow.

Table 4.1: Reactive species corresponding to the peak wavelength of gliding arc discharge

Peak Wavelength (nm)	Reactive Species
294.719	N III
309.259	OH
313.220	O III
334.851	O IV
355.383	O III
377.973	N III
389.249	N I
391.607	N II
403.390	O II
422.725	N II
651.442	N III
656.020	N II
657.291	N II
746.516	N I

4.3 Determination of Temperature

A gliding arc is a non-thermal plasma in which thermal equilibrium need to be exist. It is hard to create this condition in a laboratory, though. In this situation, the temperatures of atoms, ions, and electrons should be the same. However, each species of plasma has its own temperature if local thermal equilibrium is not present. As a result, the thermal kinetic energy of each particle is measured by the plasma temperature. The Boltzmann method, Doppler broadening, the ratio method, the Saha-Boltzmann equation, and other techniques can all be used to calculate the temperature. Temperature is determined in this case using the ratio method.

The intensity ratio between two spectral lines is included in the ratio method. Using this method, the excitation temperature is determined. The temperature is known as the electron temperature if the local thermal equilibrium persists [24].

the intensity of the spectral line is given as:

$$I_{\mathrm{ij}} = \frac{hcA_{\mathrm{ij}}g_{\mathrm{j}}n}{\lambda_{\mathrm{ij}}U(T)}e^{(\frac{E_{\mathrm{j}}}{KT})} \tag{4.1}$$

I_{ij} and λ_{ij} is the intensity and wavelength corresponding to the transition from i to j respectively, h is the Planck's constant, C is the speed of light, n is number density of emitting species, $U(T)$ is partition function, A_{ij} is the transition probability, K is a Boltzmann's constant, T is excitation temperature , g_{j} is the statistical weight of upper energy level and E_{j} is upper energy level in eV unit.

Now, taking the ratio of intensity of two spectral lines from above we get,

$$\frac{I_1}{I_2} = \frac{g_1 A_1 \lambda_2}{g_2 A_2 \lambda_1} e^{[-(\frac{E_1 - E_2}{KT})]} \tag{4.2}$$

The subscript 1 and 2 refer to the spectral lines of the same element selected. From figure 4.2 spectroscopic data is extracted from NIST database for intensity at air flow 4 ltr/min and shown in Table 4.2.

Table 4.2: Spectroscopic data of identified peaks

Wavelengths (nm)	Statistical weight g_k	Transition probability A / s^{-1}	Upper level energy Ek / cm^{-1}	Intensity (a.u.)
334.851	4	8.51 X 10^7	482666.1	1797.45
355.383	3	1.82 X 10^7	37841.84	1373.19

$$\frac{I_1}{I_2} = \frac{g_1 A_1 \lambda_2}{g_2 A_2 \lambda_1} e^{[-(\frac{E_1 - E_2}{KT})]} \tag{4.3}$$

$\frac{1797.45}{1373.19} = \left(\frac{4}{3}\right)\left(\frac{8.51X10^7}{1.82X10^7}\right)\left(\frac{355.383}{334.851}\right) Exp\left[-\frac{59.84-46.91}{KT}\right]$

T=7.98 eV

4.4 Determination of Electron Density

The number of ionized gas in an enclosed volume is called plasma density. Electron density can be calculated from relative intensity of atomic and ionic spectral lines in Boltzmann- Saha equation which is given by the equation 4.4 [25].

$$n_e = 2\left(\frac{I_1}{I_2}\right)\left(\frac{\lambda_1}{\lambda_2}\right)\left(\frac{A_1}{A_2}\right)\left(\frac{g_1}{g_2}\right)\left[\frac{2\pi m_e KT_e}{h^2}\right]^{\frac{3}{2}} Exp\left[-\frac{E_1 - E_2 + E_i}{KT_e}\right] \tag{4.4}$$

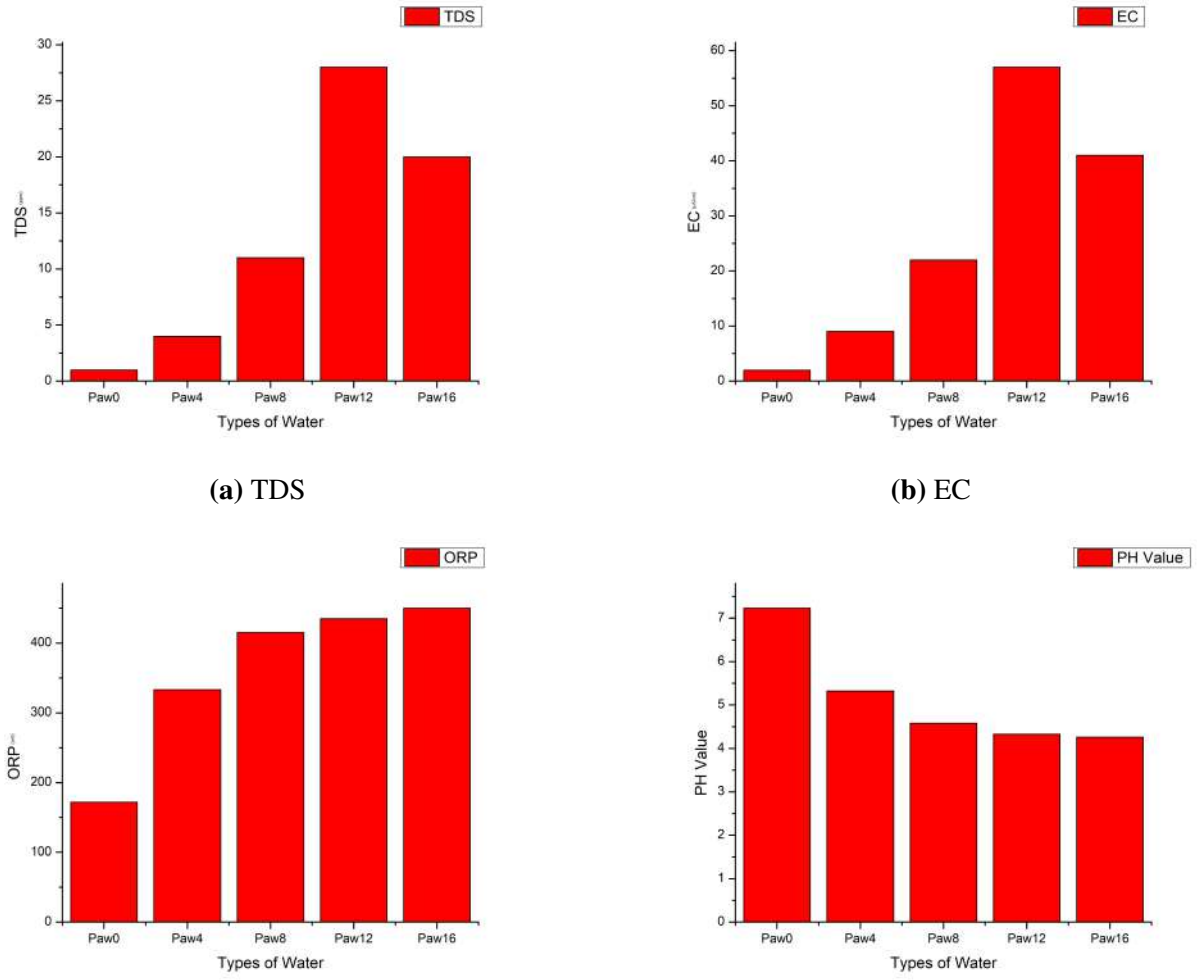
From above equation, I_1 is the intensity of the O-IV line; I_2 is the intensity of the O-III line; λ_1, λ_2 are the respective wavelengths; and g_1 and g_2 are the statistical weights of levels (1-neutral) and (2-ionized); A_1 and A_2 are the transition probabilities; respectively; m_e is the mass of an electron; T_e is the electron temperature; E is the energy of the emission level; E_i is the ionization energy of a neutral atom. From this equation we can now calculate the value of electron density as,

$n_e = 2\left(\frac{1797.45}{1373.19}\right)\left(\frac{334.851}{355.383}\right)\left(\frac{1.82\times10^7}{8.51\times10^7}\right)\left(\frac{3}{4}\right)\left[\frac{2\pi m_e \times 7.98\times1.6\times10^{-19}}{h^2}\right]^{\frac{3}{2}} Exp\left[-\frac{59.84-46.91+13.61}{7.98}\right]$

$n_e = 9.60 \times 10^{26}$ m^{-3} or 9.60×10^{20} cm^{-3}

4.5 Physical Parameter of Plasma Activated Water

The physical parameter of plasma activated water is measured from multi-probe for different time plasma exposed is shown in Figure 4.3. The pH value is decreased while increasing the gliding arc discharge exposed time but electrical conductivity, total dissolved solid, and oxidative reduction potential is going to increased due to the presence of radical nitrogen species and oxygen species provided by gilding arc discharge. Due to the atmospheric air feeder gas, the discharge contain nitrogen and oxygen species.Which are ionized due to high voltage and soluble in water.



(a) TDS **(b)** EC

(c) ORP **(d)** pH

Figure 4.3: Physical parameter for the plasma activated water for different treatment time.

4.6 Colonization and Fruiting body Appearance Period

The time duration required for the colonization was found lowest 20 days for LMS_2, LMS_3, LST_3 and more time taken by $LPAW_1$. Similarly, period required for the fruit initiation was found 25 days for five treatment and higher time duration for $LPAW_1$ is shown in Figure 4.4. The colonization period was started from 2 weeks and complete with in the 3^{rd} weeks with total of 2 replicates which cultivated by using untreated and treated mushroom spawn grain, straw, and water via cold plasma system is shown in Figure 4.5. Which is similar to the study carried out by Agun et al.(2020). The fruit initiation period of plasma treated spawn and straw was less than other but the longest time taken by the fruit initiation was by $LPAW_1$. The time duration for faster that was happened due to the plasma treatment on spawn and straw before packing.

(a)

(b)

Figure 4.4: (a) Colonization completion and (b) fruiting body initiation.

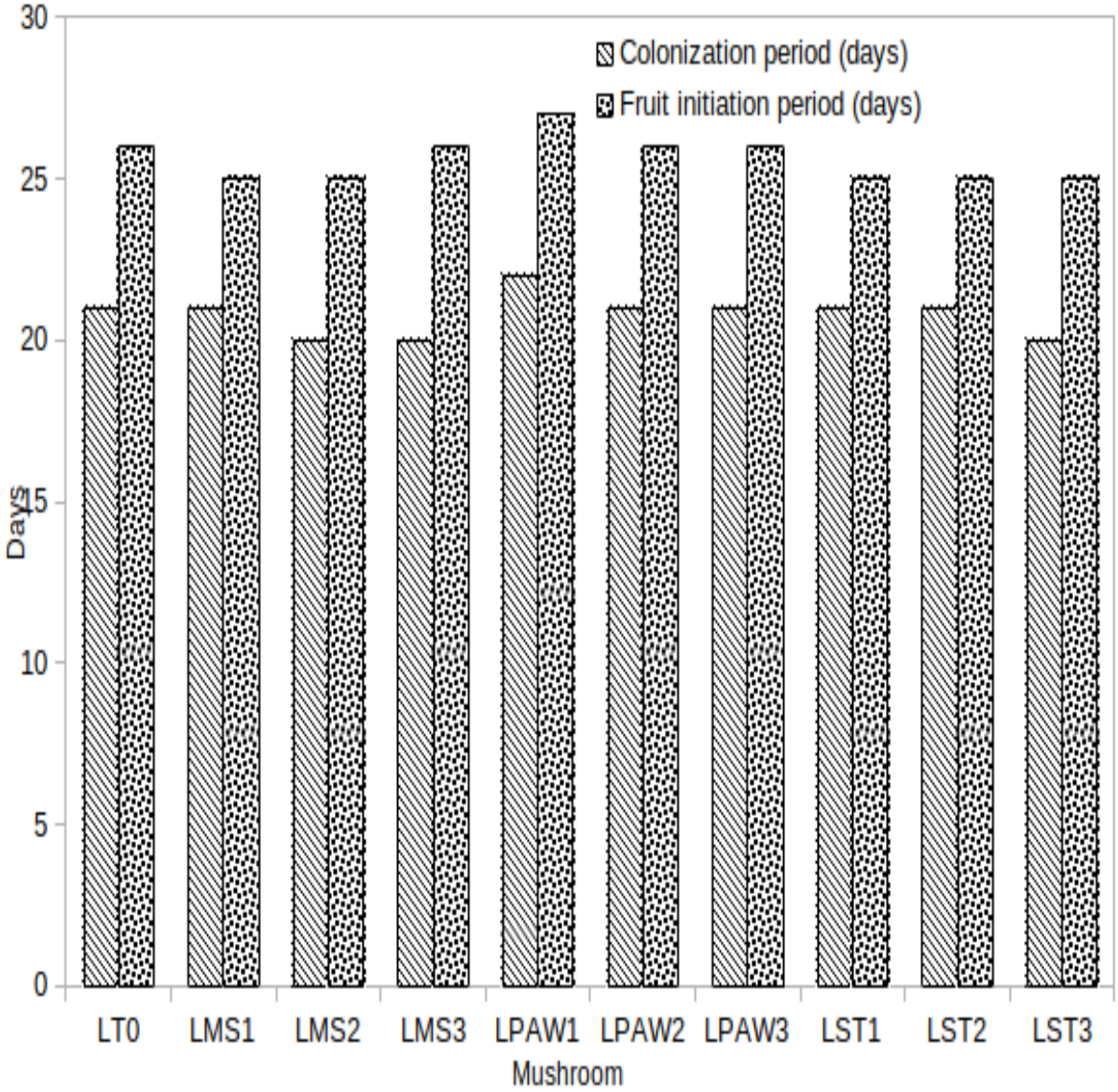


Figure 4.5: Colonization and fruit initiation day in different configuration of oyster mushroom.

4.7 Production of Mushroom

The different picture of the mushroom production in Central Department of Physics laboratory during the first and second harvesting time are presenting in Figures 4.6, 4.7 and 4.8.

(a) LMS_1

(b) LMS_2

(c) LMS_3

(d) $LPAW_1$

(e) $LPAW_2$

(f) $LPAW_3$

(g) LST_1

(h) LST_2

(i) LST_3

(j) LT_0

Figure 4.6: Pictures of mushroom before first harvesting or after 30 days of spawning.

(a) LMS_1

(b) LMS_2

(c) LMS_3

(d) $LPAW_1$

(e) $LPAW_2$

(f) $LPAW_3$

(g) LST_1

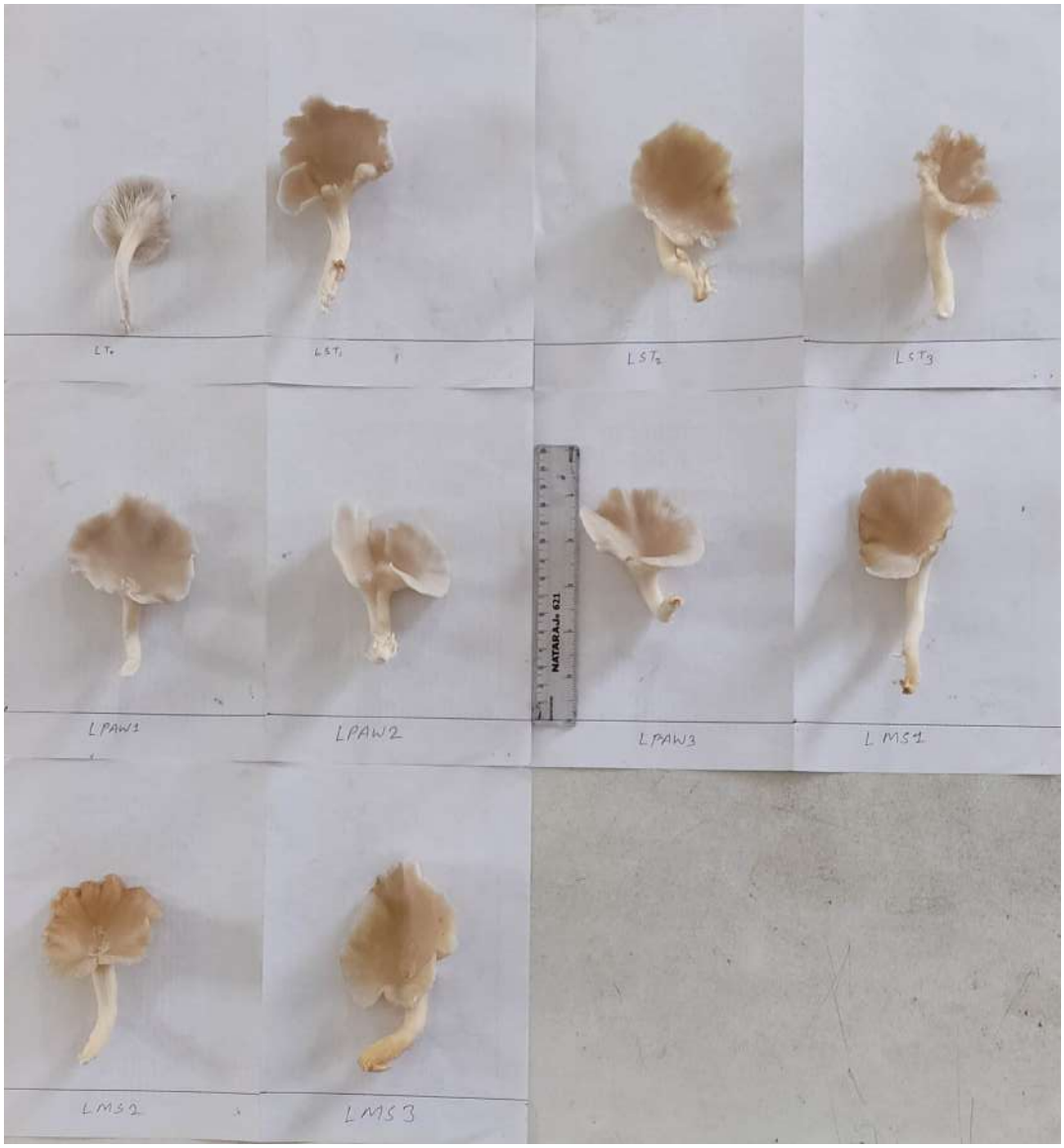
(h) LST_2

(i) LST_3

(j) LT_0

Figure 4.7: Second time fruiting body appearance of mushroom after ten days of first harvesting.

(a) Mushroom Ready for Harvesting at CDP LAB



(b)

Figure 4.8: Pictures of mushroom at second harvesting (a)before harvesting (b) comparision between different treatment after harvesting

During the period of data collection of first and second harvesting, we selected the best five samples from each treatment (category) and took the stem diameter, stem length, and cap diameter of each and also took photos of the best of one among five from each category for comparison. The first harvesting was done after 30 days of spawning and the second harvesting was after 15 days of the first harvesting period.

4.7.1 Length of Stem, Diameter of Stem and Cap

During the harvesting time, the largest fruiting body of each mushroom was selected, and its stem length, diameter, and cap diameter were compared with those of the other categories. The data was measured using a vernier caliper and best one of them from each treatment was taken for comparison as in Figures 4.9, 4.10, 4.11.

Figure 4.9: Comparison between control and mushroom spawn treated with plasma.

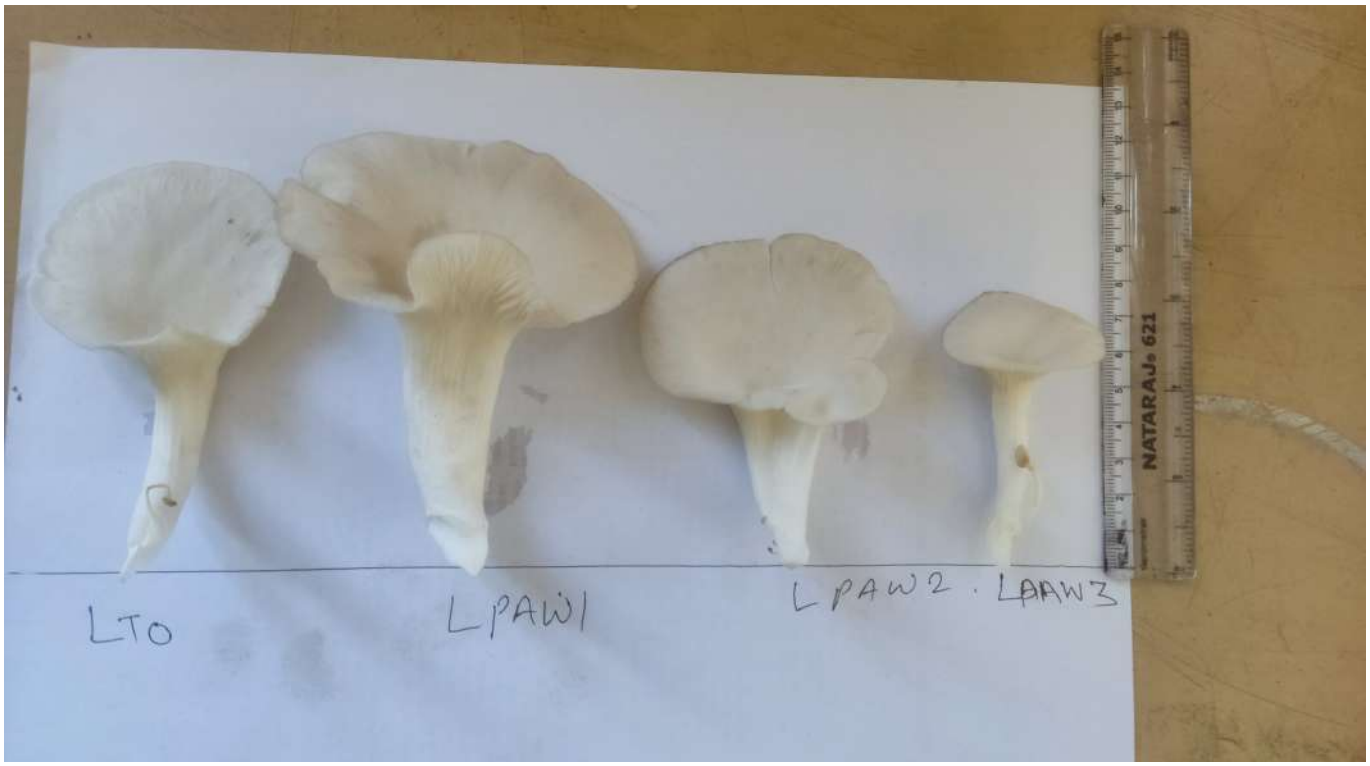


Figure 4.10: Comparison between control and mushroom that sprayed with plasma activated water.

Figure 4.11: Comparison between control and rice straw treated with plasma.

Table 4.3: Measurement of stem length (cm), stem diameter (cm) and cap diameter (cm) of various treatments.

Mushroom	Length of stem(cm)	Diameter of stem (cm)	Diameter of cap (cm)
LT_0	5.64^{c}	0.91^{d}	4.45^{c}
LMS_1	5.71^{c}	0.89^{d}	4.75^{c}
LMS_2	6.54^{a}	0.90^{d}	5.31^{b}
LMS_3	6.87^{a}	0.99^{b}	5.99^{a}
$LPAW_1$	5.76^{c}	1.22^{a}	5.51^{a}
$LPAW_2$	5.33^{d}	1.06^{b}	4.99^{b}
$LPAW_3$	5.16^{d}	0.83^{e}	4.18^{c}
LST_1	5.66^{c}	0.85^{d}	4.89^{b}
LST_2	6.13^{b}	0.95^{c}	5.36^{b}
LST_3	5.02^{e}	1^{b}	4.77^{c}
LSD Value	0.35	0.074	0.596

(Figure in a column having common letter(s) does not differ significantly at 5% level of significance.)

From the table 4.3, the length of stem was found significantly highest for the treatment LMS_3 (6.87 cm) followed by LMS_2 (6.54 cm) and LST_2 (6.13 cm).Where, the length of stem was found significantly lowest for the LST_3 (5.02 cm) as shown in Table 4.3. The diameter of stem was found significantly highest for $LPAW_1$ (1.22 cm) followed by $LPAW_2$ (1.06) and LST_3 (1 cm). However the stem diameter was found significantly lowest for $LPAW_3$ (0.83 cm). In the same way, the cap diameter was found significantly highest for LMS_3 (5.99 cm) followed by $LPAW_1$ (5.51 cm) and LST_2 (5.36 cm) and the significantly lowest for $LPAW_3$ (4.18 cm).

4.7.2 Fresh Weight and Biological Efficiency(BE)

The total fresh weight of the first yield was found significantly highest for LMS_2 (181.20 gm) followed by LST_2 (153.00 gm) and $LPAW_2$ (152.20 gm). Where, the total fresh weight of first yield of lowest significant was found for $LPAW_3$ (95.85 gm). Similarly the highest significant yield was found from second yield was LMS_3 followed by $LPAW_1$ (106.36 gm) and LMS_1 (106.18 gm) as shown in Table 4.4 and in Figures 4.12 and 4.13.

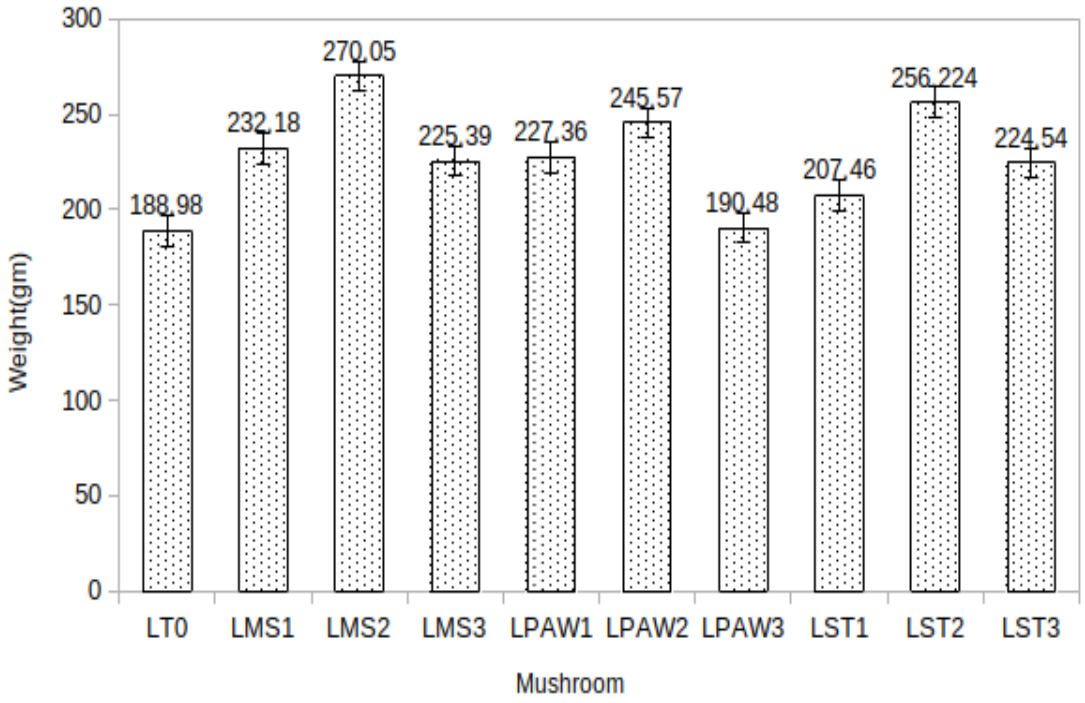


Figure 4.12: Total production of mushroom in gm.

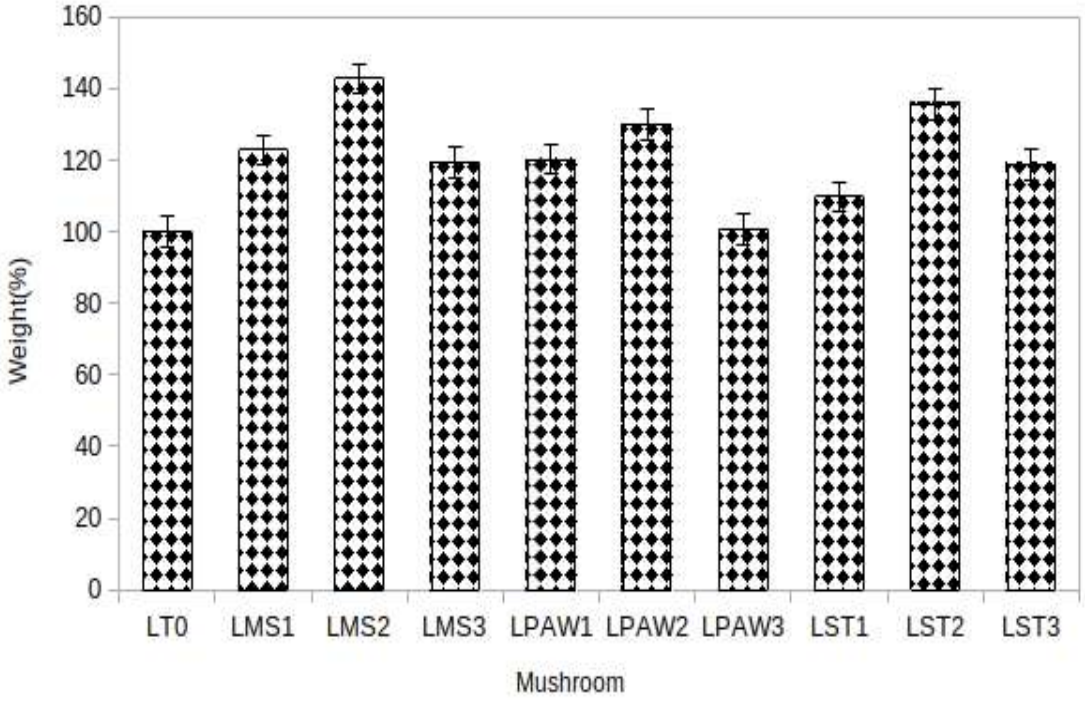


Figure 4.13: Total percentage(%) production of mushroom in gm.

From the total fresh weight of the mushroom yields and the biological efficiency the LMS_2 (270.05 gm) was found significantly highest followed by LST_2 (256.22 gm), $LPAW_2$ (245.57 gm) and lowest for untreated *i.e* LT_0 (188.98 gm) respectively as in Table 4.4.

Table 4.4: Effect of atmospheric plasma on fresh weight of first yield (gm) and second yield (gm) ,total fresh weight (gm) and biological efficiency (%) of the mushroom.

Mushroom	Fresh weight per bag (gm)			Biological efficiency (%)
	1st production	2nd production	Total production (gm)	
LT_0	128.73^{c}	60.25^{d}	188.98^{d}	94.49^{d}
LMS_1	126.00^{c}	106.18^{a}	232.18^{b}	116.09^{b}
LMS_2	181.20^{a}	88.85^{b}	270.05^{a}	135.03^{a}
LMS_3	110.00^{d}	115.39^{a}	225.39^{b}	112.70^{b}
$LPAW_1$	121.00^{c}	106.36^{a}	227.36^{b}	113.68^{b}
$LPAW_2$	152.20^{b}	93.37^{b}	245.57^{b}	122.79^{b}
$LPAW_3$	95.85^{e}	94.68^{b}	190.48^{d}	95.24^{d}
LST_1	150.30^{b}	57.16^{e}	207.46^{c}	103.73^{c}
LST_2	153.00^{b}	103.22^{a}	256.22^{a}	128.11^{a}
LST_3	145.50^{b}	79.64^{c}	225.14^{b}	112.57^{b}
LSD Value	11.72	13.92	24.35	12.17

(Figure in a column having common letter(s) does not differ significantly at 5% level of significance.)

The biological efficiency among of the different treatment was found significantly highest for LMS_2 (135.03%) and lowest for LT_0 (94.49%). For the first harvesting of fruiting body of the various cold plasma treated mushroom spawn two minutes treated is best and lowest production is for 12 minutes plasma treated with water. In second harvest of the mushroom spawn three minutes treated mushroom production is most and lowest production is for five minutes straw treated with cold plasma. Spawn treatment using cold plasma shows the mushroom fruiting body production cycle exhibit a better results when produce almost 1.5 times more compare to control spawn which is nearly same as Agun et al. (2020). similarly, for the substrates treated with cold plasma and plasma activated water used bag the production of mushroom is greater than the control spawn.

Limitation

When distilled water is added to the mushrooms that have been sterilized and grown, the mushrooms also start to die. It may be because the acidity of distilled water is higher. And due to the lack of a suitable place to do the mushroom experiment, we wrapped the mushroom inside the plasma lab with a cloth around the table and made it slightly dark. But if we experiment like this in the mushroom farm for better mushroom production, we can get even better results.

CHAPTER 5

CONCLUSION AND FUTURE PROSPECTS

Chapter 5

CONCLUSION AND FUTURE PROSPECTS

5.1 Conclusion

From the experiment of plasma treatment on *Pleurotus ostreatus* by gliding arc discharge(GAD), the time for the colonization period for plasma treated mushrooms was found to be less (20 days) than control (21 days). Similarly, the fruiting body appearance day was found to be shorter (25 days) for treated mushrooms than control (26 days) . The length of stem was found to be highest for spawn treated with plasma for three minutes (LMS_3), i.e., 6.87 cm, and lowest for substrate treated with plasma for 15 minutes (LST_3) , i.e., 5.02 cm . The diameter of the stem was found to be significantly highest for plasma activated water for 4 minutes ($LPAW_1$), i.e., 1.22 cm, and lowest for plasma activated water for 12 minutes ($LPAW_3$), i.e., 0.83 cm, and the pileus was found significantly highest for spawn treated with plasma for 3 minutes (LMS_3), i.e., 5.99 cm, and lowest for plasma activated water for 12 minutes used ($LPAW_3$), i.e., 4.18 cm. The total yield of the mushrooms from the first and second flush was found to be significantly highest for spawn treated with plasma for 2 minutes (LMS_2), i.e., 270.05 gm, and lowest for control (LT_0), i.e., 188.98 gm.

From the study, it can be concluded that spawn treated with cold plasma from gliding arc discharge among various treatments the spawn treated for two minutes is the best treatment followed by straw treatment for 10 minutes and 8 minutes plasma activated water sprayed on the oyster mushroom (*Pleurotus ostreatus*) as compared to other treatments. The thorough analysis of the impact of plasma treatments on the production of mushroom mycelium colonization and fruiting bodies that are produced as a result of this study has increased the productivity of mushroom farmers.

5.2 Future Prospects

Further studies can be done systematically modification of electrode voltage, current and by maintaining suitable temperature, humidity for the better growth and development of oyster mushroom and for other variety of mushroom as well.

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