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Spawn Treatment by Cold Plasma for Increase Mushroom Germination and Production

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Abstract. Cold plasma technology has found favour in the agricultural industry for growth stimulating by environmental friendly approach. However, there are still leaking studying of cold plasma technology on the mushroom needs. Current convectional mushrooms germination process requires long duration (~6 weeks) for fruiting to growth. Therefore, this study aims to investigate the cold plasma efficacy towards the oyster mushroom germination speed and fruiting body production. By using novel atmospheric cold plasma pen system, the mushroom spawn grains were generates towards the spawn. Atmospheric pressure with flow rate of 4, 5 and 6 SLM by considering different duration plasma exposure (0, 5, 15, 30, 45 and 60 seconds) with ~7 kV of supply voltage was supplied. The efficiency of the treatment was characterised by mushroom cultivation performance particularly on (i) mycelium growth rate and (ii) mushroom fruiting body productions. The results show cold plasma processing parameter, flow rate and treatment time absolutely influence the mushroom germination and production. CP pen system optimized at 5 SLM and 15 s presents triple production of mushroom weight and speed the mycelium growth rate (only 4 weeks) compared to control spawn grains (6 weeks). As conclusion, cold plasma pen system capability applies in mushroom industry.

1. Introduction

Human relationship with mushrooms is fascinating since they have been used both as a food and medicine for last few decades. The studies on Cultural Revolution reveal that mushrooms were an integral part of the human diet since the time when they were food gatherer and hunters[1]. The pleurotus ostreatus, known as oyster mushroom as shown in Figure 1 are edible macro fungi cultivated worldwide particularly in South East Asia, Europe, and Africa. These basidiomycetes rank second in the global mushroom production. Its popularity could be attributed to its simple and low cost production, palatability, and high biological efficiency[2]. Owing to meet the demand of growing population, the cultivation of mushroom as a source of fungi growing was proposed. It's a great opportunity for Malaysia to take advantage of the lucrative trade[3]. However, there are several boundaries facing by mushrooms entrepreneurs during the mushroom cultivations which was limited the mushrooms productions such as (i) the conventional method for cultivation the mushrooms need at least 6 weeks to grow the mushroom[4] and (ii) poor quality of spawn grain with improper treatment. Chemical treatment was found to be effective for treat the spawn due to enhancing the growth and

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yield, but might be detrimental at later stages ultimately harms the human life. Thus, these problems should be solved by using CP technology without usage of chemical. CP is an environmental friendly approach, promotes significant germination growth rate, along with control contamination and potential to provide transformative with sustainable technology interventions in agricultural industry[5]. It had been increase number of studies on seed growth enhancement and plant development using cold plasma treatment[6]. CP applications widely used in food and agricultural industry when remain works at low temperature, not exceed 60 ºC and close to room temperature[7]. Cold plasma is an alternative to avoid the use of other conventional method especially chemical process applied in the agricultural. Therefore, this study aim to bring up the CP system on mushroom industry by treat the mushroom spawn grains to enhance the oyster mushroom germination rate and its productivity.

Figure 1. The oyster mushroom (*pleurotus ostreatus*)

2. Methodology

2.1. Dielectric Barrier Discharge (DBD) plasma pen reactor

The experiment on mushroom spawn grains was carried out using a DBD-CP pen reactor as shows in Figure 2. The DBD plasma settings will be set in a fume chamber. By using air compressor (2.5HP 24L KCM), the atmospheric pressure as carrier gas with flow rates of 4, 5 and 6 SLM was generated to discharge the plasma. Five different time exposures, 5s, 15s, 302, 45s, 60s was flow into the plasma system. The DBD was generated power to the stainless steel electrode rod by supplying 7kV AC high voltage (PVM500-0.5-40kV, 20 kHz-70 kHz, max 300W). The ground electrode was wrapped using copper material. The parameter selected for spawn treatments were listed in Table 1.

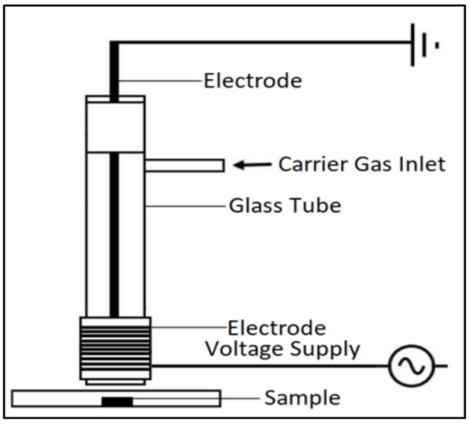

Figure 2. The dielectric barrier discharge cold plasma pen

Table 1. The parameter for spawn grain treatments.

Parameter	Values
Voltage Output (kV)	~7
Carrier gas type	Atmospheric pressure
Gas flow rate (SLM)	4, 5, 6
Duration time (seconds)	0, 15, 30, 45, 60
Spawn amounts	10 count grains

2.2. Mycelium Growth

By using DBD-CP pen system, 10 countable of spawn grains were treated for every selected parameter before inoculated into the mushroom substrate bag. For control sample, 10 countable untreated spawn grains directly placed on the mushroom substrate. The spawn grain was gathered from Saifulam Agro farm Benut, Johor, Malaysia. Then the spawning bag was placed on the mushroom house for mushroom fruiting body productions. The efficiency of the plasma treatment was examined by recorded the growth rate of the mycelium (Figure 3) every week. The mushroom productivity measured by weighing the first cycle of the harvested mushroom fruiting body productions (3 replicates for each parameter). They were measured using weighing scale commonly used in the lab.

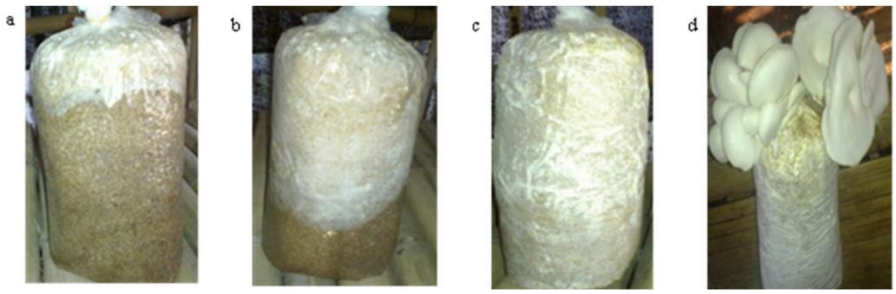

Figure 3. Mycelium growth process: (a) after 2 weeks: (b) 3 weeks: (c) 4 weeks; (d) fruit body after 6 weeks[4]

3. Results

3.1. Mycelium Growth

Figure 4 present the mycelium growth with total of 3 replicates which cultivated by using untreated and treated mushroom spawn grain via cold plasma system. The graph shows 97% all the mycelium started to growth on week 2 and completed the mycelium growth on week 6. The atmospheric pressure flows on 5 SLM has faster mycelium growth followed by 4 SLM and 6 SLM. Focused on week 4th, flow rate of 5 SLM was successfully complete the mycelium growing process (20 cm) while the control spawn just growth its mycelium for 14 cm height. The control spawn required 6th weeks to complete the mycelium growth[4]. The flow rate of atmospheric air pressure and treatment time had been related to greater mycelium growth via cold plasma treatment. Therefore, can be seen, mycelium growth by cold plasma based on treatment time shows 15 s is the faster growth compared to control spawn and others duration time (5s, 30s, 45s & 60 s). This because the cold plasma plays essential roles in a broad spectrum of developmental and physiological processes in mushrooms, changing spawn surface properties and stimulating spawn mushrooms germination[8]. Treatment time 15 s was impressive compared to others because too long expose to plasma will decreased the plant growth and vitality. Results shows cold plasma treatment depends on flow rate and duration as processing parameter. Therefore spawn treated using cold plasma by parameter of 5 SLM with 15 s shows 50% performance compared to control spawn grain.

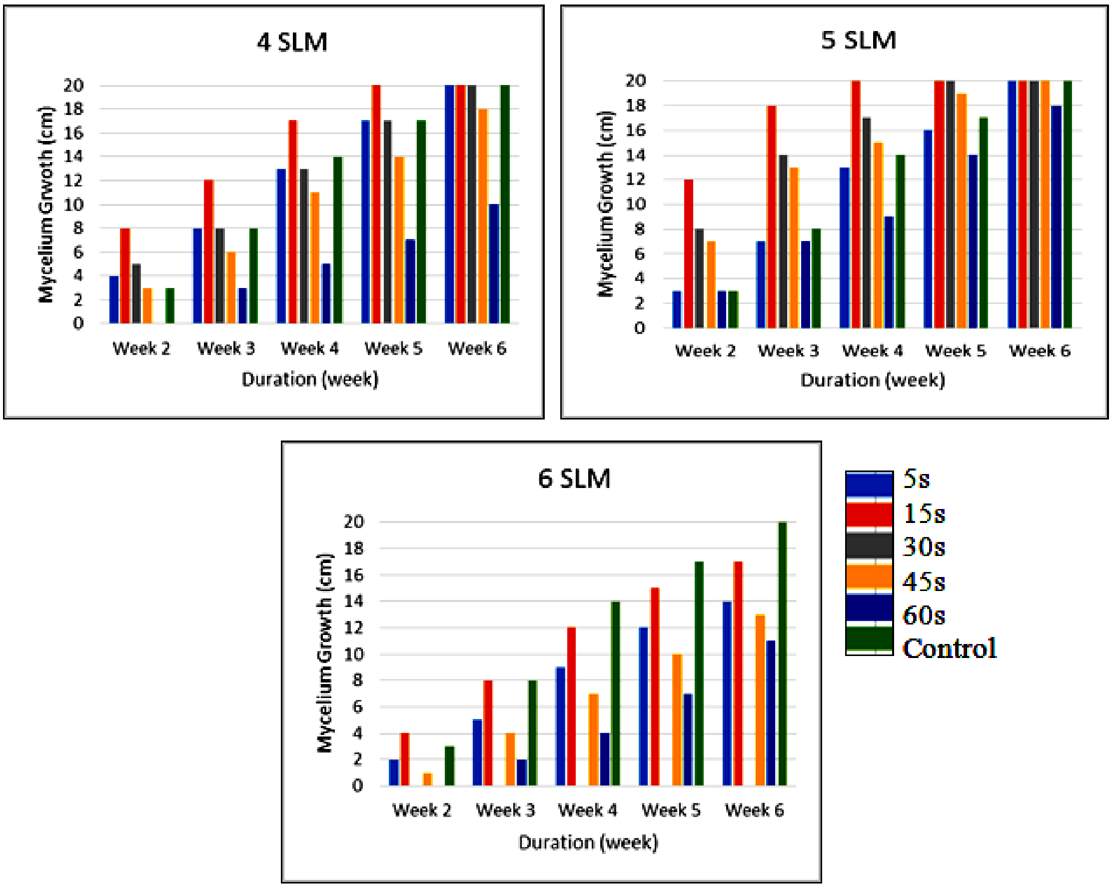

Figure 4. Mycelium growth at different flow rates: 4 SLM, 5 SLM and 6 SLM

3.2. Production of Mushroom Fruiting Body (Mushroom Weight)

Figure 5 shows quantitative results of weighing mushroom fruiting body (3 bag logs), harvested on the first cycle of the mushroom cycles. Data illustrated treated spawn grain with 5 SLM flow rate and 15 s of duration time able to produce 517.99 g of fresh fruiting body compared to control spawn, which capable produce 150 g. This present the cold plasma treatment spikes 3 times performance compared to control spawn. However, 6 SLM treatment shows low productivity when produce below than 50 g. This was related with the ionization process during the plasma bombardment. To high expose to plasma discharge will reduce the mushroom productions. The gas flow rate of atmospheric pressure was influence the operation of discharge, the retention time of reactive species in the spawn, as well as the mass transfer process of reactive species. By increasing the flow rate, the collision and reaction possibilities of reactive species can be enhanced. Whereas, a high flow rate would in turn lead to a decrease in the retention time of reactive species in spawn, which is unfavourable to the growing process. According to Wang et al., (2014), keep increasing of air flow rate was reducing the remediation performance[9].

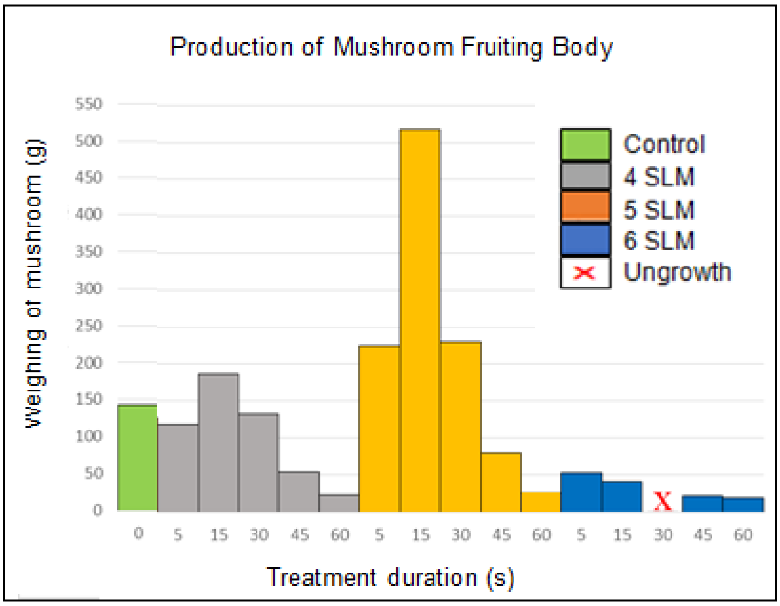

Figure 5. Quantity 1st cycle harvested mushroom by treated and untreated spawn

Figure 6, present the graph of comparison between control and 15 s treated spawn grain using cold plasma from 1st cycle until 7th cycle productions of mushroom fruiting body. Results identified that 15s of fruiting body productions show higher production weight with 1802 g of total weight compare with control spawn production only produce 799 g of total weight. Spawn treatment using cold plasma shows the first three mushroom fruiting body production cycle exhibit exceptional results when produce almost 2-5 times more compare to control spawn. Duration of the spawn grain treatment mainly influence the plasma process. According to Bourke et al., (2018), the suitable duration time for seed treatment is between 5 seconds to 30 minutes[10]. Higher treatment times resulted in lower imbibition and germination values[5]. Exposed the plasma directly to a sample, causes etching and erosion the surface are probably the most dominant processes[11]. The sample coat is then modified by cold plasma treatment and probably the seed surface loses its water-permeability character, consequently, the dormancy is more likely to break down under the influence of moisture. Therefore we can see, 60 s of treated spawn decrease its mushroom weight production.

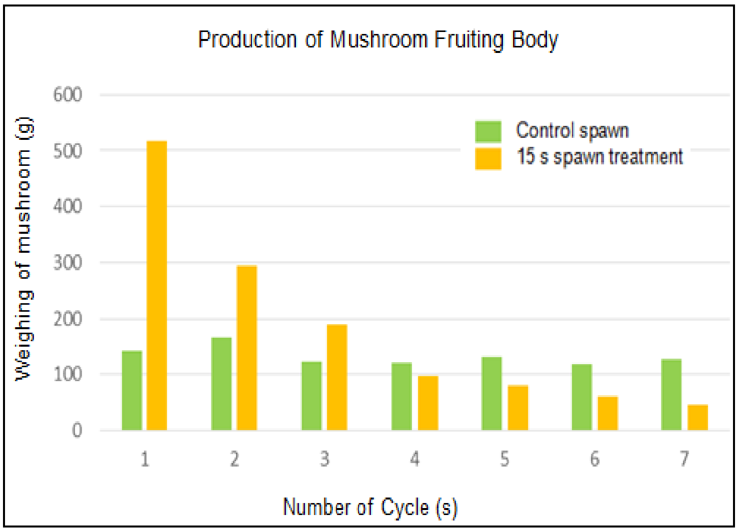

Figure 6. Production of mushroom fruiting body for 5s treatment time and control spawn

4. Conclusions

This study enriches the information to the knowledge of mushroom production by treated spawn grain. Results show that by atmospheric cold plasma pen treatment, 50% rapidly mycelium growth rate was achieved and triple production of mushrooms can be observed after cultivated by using treated spawn grains via environmental friendly approach, a cold plasma system. 5 SLM with 15 s was selected as optimized parameter when demonstrated the best mushroom cultivation performance. This optimized parameter related with transferring atmospheric pressure gas and responsible for the initiation and propagation of plasma reactions on the spawn grain. Thus this study contributed in a systematic investigation of the influence of plasma treatment on mushroom mycelium growth and fruiting body productions cultivation process and the impact of these findings help mushroom entrepreneurs increase their productivity.

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