

Effects of High Voltage Stimulation and Oxygen Rich Fine Bubble (FB) Water on Cultivated Shiitake Mushroom in Thailand

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Abstract— In recent years, high voltage plasma (HVP) and fine (micro/nano) bubbles (FB) have attracted great concern in both agriculture and aquaculture. It was reported that the total weight of mushrooms increased by 1.3 - 2.0 times after applying high voltage compared with control. In addition, FB can promote the growth rate of plants, fishes and mammals. In this study, the effects of high voltage stimulation and oxygen rich FB water on cultivated shiitake mushroom in Thailand was studied. Results show that combination of high voltage stimulation and O₂ rich FB water significantly promoted the total weight of shiitake mushroom by 1.8 times compared with the control. Consequently, these innovative methods are found to be potentially effective tools for increasing the mushroom yields.

Keywords—High voltage stimulation, fine bubble, shiitake mushroom, fruit body formation

I. INTRODUCTION

Shiitake mushroom (*Lentinula edodes*; *L. edodes*) is a symbol of longevity in Asia because of their health promoting properties, aroma and good taste. Furthermore, regular shiitake mushroom consumption resulted in improved immunity [1]. For these reasons, Thai government has been, and is encouraging farmers to grow shiitake mushroom, especially in northern area of Thailand, since the climate (winter temperature, 18 - 25 °C) is suitable for the mycelial growth of shiitake and also decreases the pest fungi contamination rates [2].

In Thailand, we have some traditional method as shown in Fig. 1 for stimulating the fruiting yield of shiitake mushroom by beating the top of substrates with a sandal after cutting the upper plastic bags, and then providing lower temperature and high humidity atmosphere. However, the domestic production of shiitake mushroom has not met the total demand yet in some season, so the substantial quantity of mushrooms has been occasionally imported [2]. Therefore, Thai farmers still need an alternative effective technique to drastically increase the yield, which will result in additional values and improved competitiveness in the global ASEAN market.

Meanwhile in Japan, there are many research groups started using an artificial lightning to stimulate various mushrooms [3-12]. Eight out of ten Japanese mushroom species were reported to have a doubled crop yield by applying high voltage stimulation compared with control, and can reduce the necessary chemical fertilizer by 90% compared with the traditional cultivation method in Japan [6].

Since 2016, we also started in northern of Thailand to survey the effects of high voltage stimulation (HVS) on the expensive edible Thai mushrooms by using a high voltage stimulation device called “Raizo” (GM100; Green Techno Co., Ltd., Japan) imported from Japan as shown in Fig. 2.

The preliminary study showed that it can accelerate the growth of cultivated shiitake mushroom (*Lentinula edodes*) and Hed kra dang (*Lentinus polychrous* Lev.) by 1.8 times compared with the traditional Thai method [13].

In addition, fine bubble (FB) technology is being studied and found to be very effective to physiological activity of living organisms [14-17], due to their remarkable property such as highly efficient gas solubility, larger specific surface area and negative electronic charges on the bubble surface which may promote the plant growth [18-20]

Previous studies showed that air-microbubbles promoted the growth rate of leaf lettuce compared with air-macrobubble



Fig. 1 Thai traditional stimulation technique.



Fig. 2. Artificial lightning device called “Raizo”.

[14], and air-nanobubble significantly promoted the height, length of leaves and aerial fresh weight of *brassica campestris* compared with normal water [15]. Moreover, cucumbers planted under high dissolved oxygen (DO) concentration can increase area and weight of leaves [20]. However, the effects of fine bubble in the shiitake mushroom growth have not been reported so far.

The objective of this study was to examine the effects of combination of high voltage stimulation and oxygen rich fine bubble (ORFB) water in cultivated shiitake mushroom in Thailand, in order to explore its potential for promoting the growth rate of shiitake mushroom by comparing the growth rate between applying ORFB water and tap water after electrical stimulations.

II. METHODOLOGY

A. Shiitake bag cultivation

The strain of fruiting type was *L. edodes* strain no. 4 (recommended strain of Department of agricultural (DOA), Thailand). The shiitake bags were prepared by using para rubber sawdust mixed with 8% rice bran, 1% of sugar, 0.5 % of lime, 0.5 % of gypsum and 0.2 % of magnesium sulphate, mixed thoroughly with mixing machine, and water was added to make a moisture content about 65%.

The mixture was placed in heat-resistant plastic bags (10-cm dia. x 15-cm depth cylindrical) and then bags were pasteurized by steaming at 90 °C for 5 hours (Local Thai Made model). When sterilization was completed, the bags were moved to a cooling room and cooled to the ambient temperature at 30 °C.

Grain spawn was inoculated into the cooled bags, and several grains were poured into the bags and wrapped again with a cotton plug and newspaper. After inoculation with grain spawn, bags were transferred to incubate at uncontrolled incubation room for 4 months, and during incubation in the dark room, the shiitake fungi permeated and degraded the substrates; the shiitake mycelium virtually knitted the sawdust medium together.

B. Stimulation method

After incubation for 4 months, the bags were opened by removing the plastic necks and the upper part of the bag in cut. This experiment was carried out at Rajamangala University of Technology Lanna (RMUTL), Thailand.

A high voltage generator called "Raizo" (Raizou; GM100) based on Cockcroft-Walton circuit was chosen for stimulating the fruiting yield of shiitake mushroom. Fig. 3 shows waveform of 14 kV applied voltage through the mushroom bed and the pulsed width is 420 ns. The impedance of mushroom bed is 650 ohm. The shape of applied voltage waveform shows almost the same as Fig.3, Fig.1. and Fig. 2. in ref. [10], [11] and [12], respectively.

Fig. 4 shows a photo of experiment setup used for applying high voltage stimulation to shiitake mushrooms. The substrates were placed on the mobile shelves. A ground wire from main unit of the equipment and mobile shelves were connected to a ground rod which inserted into soil. The electrode was placed near the top of the substrate with the distance around 1-7 cm which depend on the range of applying voltage (20 kV – 50 kV) which showed voltage value at the

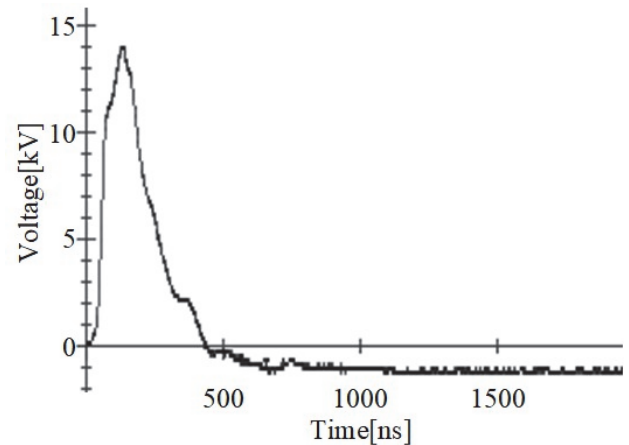


Fig. 3. Waveform of output voltage of Raizo.



Fig. 4. High voltage stimulation on mushroom.

monitor of the Raizo equipment. Each substrate was applied by high voltage for 10 seconds.

In this experiment, three groups were applied as an attempt to induce fruit body of shiitake mushroom by using different stimulation methods. 1) no stimulation (control), 2) beating on the top of substrate with a sandal continuously for 10 seconds and 3) high voltage stimulation method by using Raizo equipment.

After stimulation, all substrates were kept in a 3 m × 4 m controlled farm (average temperature = 19 °C, average humidity 69.4%), and studied the effects of oxygen rich fine bubble in the growth rate of shiitake mushroom. Substrates were separated in 2 groups.

Table I shows stimulation methods and water types pouring onto the substrates (50 substrates for each treatment).

Treatments of 1-6 were applied by tap water (DO = 5 mg/L). Oxygen fine bubbles were generated using a pressurized dissolution method by RMUTL – KVM20 (20 L/min at 4.5 bar exit pressure) to increase DO level to 20 mg/L, and were applied to treatments T7, T8, T9. Moreover, DO concentration was measured by Horiba Multiparameter Water Quality Meter, U-54G.

All the treatments were supplied by water twice a day onto the top of substrates (in the morning and evening before collecting the mushrooms). The fruit bodies were harvested

TABLE I
TREATMENTS, STIMULATION METHOD AND WATER CONDITION
POURING ON THE SUBSTRATE

Treatment	Stimulation method	Water type	DO level (mg/L)
T1	No stimulation (control)	Tap water	5
T2	Beating with 4.9 N for 10 sec.	Tap water	5
T3	20 kV for 10 sec.	Tap water	5
T4	30 kV for 10 sec.	Tap water	5
T5	40 kV for 10 sec.	Tap water	5
T6	50 kV for 10 sec.	Tap water	5
T7	20 kV for 10 sec.	O ₂ FB water	20
T8	50 kV for 10 sec.	O ₂ FB water	20
T9	No stimulation	O ₂ FB water	20

*Stimulation was applied only once per flush

** Water was poured onto substrates twice a day.

every day when veils just detached from stipes. Their weights were, then, measured to obtain total weight. Statistical analysis was subjected to analysis of variance followed by ANOVA. Least significant difference (LSD) test ($\alpha = 0.05$) was used to evaluate significant treatment differences.

III. RESULTS

A. Yield of *L. edodes*.

In the 1st flush, we found that small white primordia appeared 3 days after stimulation for all treatments even in control group (data not shown). Since it is possible that all substrates can contain a lot of mycelial which can be fully developed with rich nutrition in the substrates, the total weights of the shiitake mushrooms were not much different among all treatments.

However, in the 2nd flush, we observed that shiitake mushrooms appeared faster for beating method (T2), and high voltage stimulation technique with and without FB water (T3-T8), compared with control (T1) (data not shown).

Fig. 5 shows pictures of the 3rd flush of shiitake mushrooms on decreased nutrition's substrates. It was found that plenty of mushrooms were seen only in T2-T8 treatments. In addition, 20 kV + O₂ FB water treatment (T7) was found to produce the highest total weight (1,463 g), whereas 50 kV+ O₂ FB water (T8) 1,389 g, and beating treatment (T2) 1,007 g, respectively. The yield of control group (T1) was found very poor, i. e. , only 27 g, and O₂ FB FB without stimulation (T9) gave 134 g as shown in Fig. 6.

In addition, after collecting mushrooms for 175 days (Fig. 7 and Table II), it was found that there were no significant differences among beating, high voltage stimulation, and combined HVS + O₂ FB water treatments.

However, when compared with control these 3 methods revealed significant differences at $p < 0.01$. Moreover, fruit body yields were found to increase to 170 - 180 % under 3 method stimulations compared with control.

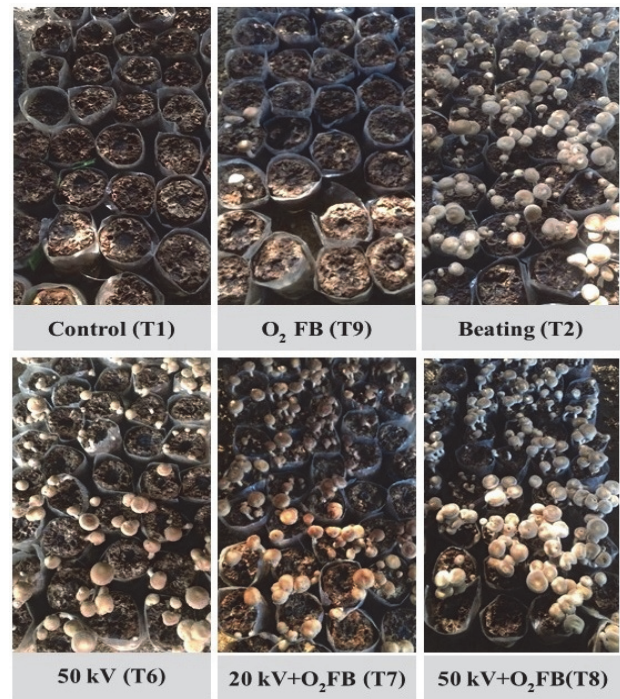


Fig. 5. Photograph of the 3rd flush of cultured shiitake mushroom after stimulation for 3 days.

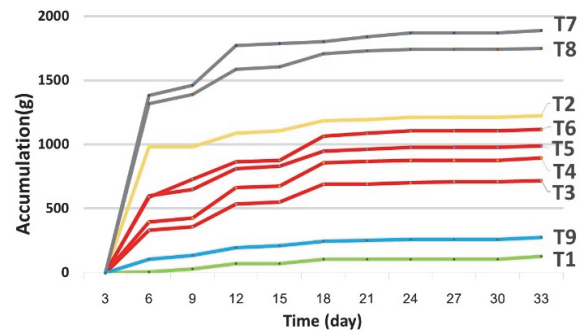


Fig. 6. Diurnal variation of accumulated weight of fruitbody formation in the 3rd flush of shiitake mushroom.

IV. DISCUSSIONS

Although, the mechanism of this yield increase is not clear, but there seem some factors that could promote fruiting in shiitake cultivation, such as temperature fluctuation, high humidity, soaking, removal of CO₂ and physical shocks [21].

Farmers generally know that physical shocks such as beating on the top of the substrate or cutting the substrate by sharp knife can promote mushroom fruit bodies which are generated from cracks in hyphae, and high voltage electrical shocks can do the same.

From the experiments, 3 main points are to be discussed; 1) Beating on the substrate and high voltage stimulation both resulted in triggering fruit body formation when compared with control. However, both methods resulted in different ways, namely, beating resulted in triggering fruit body formation during 3-8 days after stimulation and then

TABLE II
TOTAL NUMBER AND TOTAL WEIGHT OF SHIITAKE MUSHROOM FOR VARIOUS TREATMENTS

Treatment	Stimulation method	Water type	Total number	Total weight (g)
T1	No stimulation (control)	Tap water	478 e	6249 e
T2	Beating with 4.9 N for 10 sec.	Tap water	911 bc	11453 b
T3	20 kV for 10 sec.	Tap water	837 cd	10650 c
T4	30 kV for 10 sec.	Tap water	760 bc	11188 c
T5	40 kV for 10 sec.	Tap water	982 b	11056 c
T6	50 kV for 10 sec.	Tap water	909 bc	10926 c
T7	20 kV for 10 sec.	O ₂ FB water	864 bc	11373 b
T8	50 kV for 10 sec.	O ₂ FB water	742 c	10305 a
T9	No stimulation	O ₂ FB water	779 de	8028 d
F-Test			**	**
LSD			0.825	2.004
CV			51.1%	56.6%

** = highly statistical significant difference

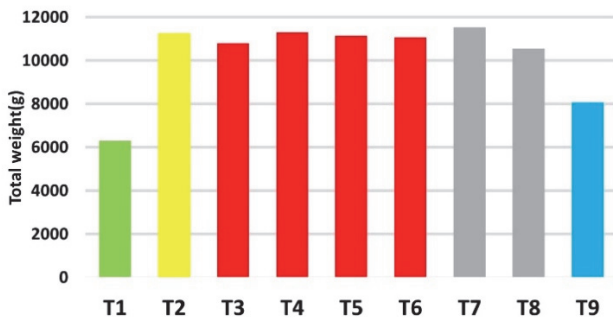


Fig. 7. Total weight of cultured shiitake mushrooms collected for 175 days.



Fig. 8. Shiitake mushroom farm in Wiang Pa Pao district, Chiang Rai province.

mushroom fruiting was stopped, whereas high voltage stimulation resulted in longer period of fruiting. Possibly, electrical pulses not only cause cracking in hyphae [3, 6] but also activate enzymes to develop mushroom fruit bodies [22]. In order to compare both physical shocks, Scanning Electron Spectroscopy (SEM) is required to acquire scientific and qualitative information, and this will be left in the future study.

2) At the 3rd and 4th flushes on the nutrition decreased substrate we found that only applied rich oxygen FB could not significantly promoting the growth rate of mushrooms because the substrate is needed stimulation for inducing fruiting yield. However, the total weight of shiitake mushrooms after applying HVS+O₂ FB (T7) was found higher than control (T1). We speculate that high voltage has made physical damages and resulted in plentiful development of mushrooms fruit bodies. In addition, the larger specific surface area of FB with highly dissolved oxygen level and negative electronic charges on the FB surfaces may promote growth. This is because negative electronic charges on FB surfaces may help primordia absorb positively charged nutrients [23].

3) Moreover, rich oxygen FB water may reduce the level of CO₂ during fruiting which tends to prevent the elongation of stems, and to slow cap enlargement caused by high concentrations of CO₂ [2,24]. In other words, high voltage stimulation can break hypha and cause promoting fruiting in shiitake cultivation and O₂FB can help primordia absorb the nutrition in the substrates.

In introducing high voltage stimulation (HVS) and oxygen rich FB water treatment to Thai mushroom farmers, there are some concerns.

One is, in a relatively huge mushroom farm (6m×12m area), holding 8,000 mushroom bags, the HVS method will take longer time for stimulation in case by hands, and the process will be more complicated compared with beating methods (Fig. 8). The other is, farmers always concern about higher cost that they have to invest in their business. These concerns call for development of compact, cheap and friendly user-oriented high voltage devices, as well as easy connection with an automatic supply of oxygen rich FB water.

V. CONCLUSION

In this study, following conclusions were drawn;

- Combination with HV stimulation and O₂ rich FB water can promote the growth rate of shiitake mushroom compared with control.

- The total weight of mushrooms by a very traditional Thai method is found also higher than control by 1.8 times.
- A compact, less-expensive and user-friendly high voltage device is rather mandatory to develop to meet farmer's requirements.
- In the future work, combination with Thai traditional method and easily operable automatic oxygen-fine bubble generators may be a promising technique for Thai farmer, resulting in additional values and improved competitiveness in the global market.

ACKNOWLEDGMENT

This study was supported in part by Hands on scholarship from Rajamangala University of Technology Lanna (RMUTL). We thank Center of Excellence of High Voltage, Plasma and Micro/Nano Bubbles Applications to Advanced Agriculture and Aquaculture, RMUTL for supporting "Raizo device" and Department of Agriculture, Chiang Rai Horticulture Research Center for sample preparation. RN thanks to the advices of Katsuyuki Takahashi from University of Iwate and the electrical experiment effort of Chanchai Dechthummarong from RMUTL.

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