Preliminary study on organic non-environmental controlled cultivation of edible fungi in Hong Kong

Introduction

Hong Kong is located at the southern tip of China. It is a typical subtropical marine climate with obvious seasonal changes. The annual average maximum and minimum temperatures are 28.8°C and 16.3°C respectively, and the temperature difference between day and night is within 10°C. Most popular edible fungi are grown in temperate zone, and the optimum cultivation temperature is generally between 5°C and 25°C. In this temperature range, these edible fungi are well development and growth in both stages of mycelium or fruiting bodies. By this reason, most edible fungi manufacturers use environmental control equipment in large-scale cultivation, so that edible fungi can have stable yields throughout the year. Examples include *Flammulina* and *Pleurotus eryngii*, which require lower growth temperatures. Some fungi producing areas are able to produce high-priced edible fungi, such as *Morchella* in Yunnan and Sichuan, and *Hericium* in Central China, due to their unique geographical and climatic conditions. The maximum daily temperature in Hong Kong from June to September is above 30°C. It seems impossible to cultivate these edible fungi in Hong Kong in the absence of environmental control.

The use of environmental control mode to manage edible fungi cultivation seems to solve the problem of growth of edible fungi at any time. This type of production mode consumes a lot of energy to create the optimal conditions for production, and at the same time, they use the optimal culture formula with the highest yield obtained through research and cultivation, even adding chemical synthetic hormones to ensure that they get the highest yield. In today's era of efficiency, it is understandable, but in fact, this model is also applied to the production of large-scale plant crops today, but recent studies have shown that although this type of production model can produce a large number of crops efficiently, it leads irreversible damage to the environment. For example, the trace elements of the land are depleted, the balance of soil nutrients required for various plants is destroyed, the ecological communities of the natural microorganisms are also destroyed, and the carbon storage capacity of the natural soil are largely affected.

Although humans can use their ingenuity to apply a large amount of cheap synthetic fertilizers or trace elements to the cultivated soil. These fertilizers are mostly water-soluble substances. Although they are easy to release and allow plants to absorb, they are also more likely to be lost. To meet the needs of a mass production of crops, resulting in excessive waste and water pollution, and this large-scale farming will discharge a large amount of greenhouse gases, leading to global warming. More importantly, studies have shown that the crops obtained from such farming have much lower nutrients than the crops obtained by traditional farming methods. Another example is the hydroponic vegetable in urban factories, which seems to be a scientific way of saving resources, but the problem is that this method of forcing plants to grow quickly resulted in even less nutrients than the above-mentioned land growing crops using chemical fertilizers. Factory-style edible fungi cultivation is similar to the production method of hydroponic vegetables, and whether the nutritional value is sufficient is still a question.

World agriculture has gradually turned to the direction of sustainability, environmental protection and regeneration. In recent years, the Hong Kong government has invested more resources to support local sustainable agriculture, including the cultivation of edible fungi. To become an environmentally-friendly smart city driven by technology, Hong Kong must take into account the problem of dense population and serious shortage of land. It seems unrealistic to use a large amount of land for agricultural development. Cultivation of edible fungi does not require occupation of agricultural land, and can be carried out in almost any space in the city. If energy conservation and environmental protection factors are considered, community recycling materials (such as food waste such as kitchen waste and bean dregs) will be used as culture materials. In response to the climate of Hong Kong, selection of suitable edible mushroom species to grow in an area without conditional control and the area who have temperature control or have temperature regulation throughout the year, such as shopping malls and offices, will result in significant cost savings. General implementation methods: (1) medium preparation and inoculation in one place; (2) distribution to various sites (including non-circular control or temperature-regulated places) for mycelial growth and mushroom management; (3) picking, Collect, transport or sell.

In this report, we discuss the cultivation of the four kinds of fungus species in the farm of Mushroom Initiative (TMI), Tai Po, Hong Kong. It is a site without environmental control. It gives suggestion to the selection of species for eco-friendly cultivation in different seasons in Hong Kong by analyzing the monthly data collected in 2017.

Materials and methods

1. Edible Fungi Species

Pleurotus ostreatus (平菇) species (Suyin 6, H2, Huiping 03 and Heiping 410), *Pleurotus geesteranus* (秀珍菇) species No. 9, *Ganoderma lucidum* (赤芝) species (Meida BG and Tianzhi SG) and *Agrocybe aegerila* (茶樹菇) species Chaxin AS1 were obtained as primary cultures in PDA test tubes from the Centre of Fungus, Huazhong Agricultural University (HZAU).

2. Materials of growth media

The saw dusts of pine log were obtained from Wong Chiu Ki wood factory Ltd (Kam Tin, Hong Kong). The coarse wooden chips were prepared by breaking down the branches of litchi or other tree species collected in Pak Ngau Shek or donated from Highways Department of Hong Kong. Both saw dusts and wooden chips were stored in outdoor for at least half year before use. The organic soybean dregs were obtained from the store, A Po Tofu Fa (Tai Po, Hong Kong). Coffee grounds were collected from Starbucks. Australia raw sucrose and lime powder were purchased from 7S sugar Ltd. (Tuan Men, Hong Kong) and Chan Kee Store (Tai Po, Hong Kong) respectively. Kitchen waste was donated by the Kadoorie Farm. Organic Potato, glucose and agar were purchased from City Super Store (Hong Kong).

3. Preparation of mushroom culture

3.1 Cultivation of *Pleurotus ostreatus*, *Pleurotus geesteranus* or *Ganoderma lucidum* with bean dregs formula (BDF)

The formula of 1 kg solid medium BDF contains 390 g (wet weight) of bean dregs, 295 g (dry weight) of saw dusts, 290 g (dry weight) of coarse wood, 10 g (dry weight) coffee grounds, 10 g lime powder, 5 g raw sucrose, 60% moisture, pH 7.0. Then about 190 g of TCM1 was packed into a 250 ml glass bottle and a tunnel of 2 cm in diameter from top to the bottom was made in the central of the solid medium for inoculation. The bottle was then sealed with plastic film and sterilized by autoclave (121°C, 60 min).

10 g of secondary culture of *Pleurotus ostreatus*, *Pleurotus geesteranus* or *Ganoderma lucidum* was transferred and inoculated into the sterile BDF bottle in a biological safety hood. The culture was sealed with a clean paper towel and incubated in an insect isolated dark room without any environmental control until the bottle was full of mycelia.

For fruiting body development, the bottle were then transferred and incubated in an insect isolated shed with mosquito net. The shed was supplied with about 30% scattered light without temperature control. Water was sprinkled to maintain the humidity (60-80%). The fruiting bodies were harvested in appropriate size.

3.2 Cultivation of *Agrocybe aegerila*

The formula of 1 kg solid medium AAM contains 291 g (wet weight) of bean dregs, 340 g (dry weight) of saw dusts, 339 g (dry weight) of coarse wood, 10 g (dry weight) coffee grounds, 10 g lime powder, 10 g raw sucrose, 60% moisture, pH 7.0. Then about 190 g of AAM was packed into a glass bottle and a tunnel of 2 cm in diameter from top to the bottom was made in the central of the solid medium for inoculation. The bottle was then sealed with plastic film and sterilized by autoclave (121°C, 60 min). The inoculation and growth condition were the same as the method described in 3.2.

Results and discussion

1. Formula development

Formula BDF and AAM described in material and method section were developed by varying the ratio of soybean dregs to wood to give the highest bioconversion rate of four selected edible fungus species. The experiment was carried out in February of 2017 for mushrooms species, and in May 2017 for *ganoderma* species. 100-bottle-cultures of each species were used in calculation. The results are shown in table 1. It shows that the ratio 4:6 (soybean dregs: wood) is the most suitable for the growth of *P. ostreatus*, *P. geesteranus*a and *G. lucidum*, while 3:7 (soybean dregs: wood) is the most suitable for the growth of *A. aegerila*.

Table 1. The bioconversion rate of individual species in formula development.

	Bioconversion rate obtained by formulating with different soybean dregs to wood ratio								
	1:9	2:8	3:7	4:6	5:5	6:4	7:3	8:2	9:1
P. ostreatus	ND	36.13	54.41	70.75	62.82	53.26	44.42	31.32	29.53
P. geesteranus	ND	24.49	36.89	47.97	42.96	36.11	30.13	21.32	20.67
G. lucidum	ND	10.78	15.53	22.28	17.53	13.14	ND	ND	ND
A. aegerila	ND	31.34	55.12	30.26	25.76	22.31	13.19	ND	ND

The species used in the experiments were Huiping 03, No.9, Meida and Chaxin AS1 for *P. ostreatus*, *P. geesteranus*, *G. lucidum* and *A. Aegerila* respectively. ND represents no mycelia growth or fruiting body development.

2. Weather condition in 2017

The monthly mean daily air temperature and mean relative humidity variation in 2017 (data obtained from the Hong Kong Observatory) are shown in Fig. 1. It provided important reference for the selection of the species cultivated in different seasons (or months). Table 2 shows the temperature for fruiting body development of the edible fungus species cultivated in TMI. The monthly variation of the mean relative humidity ranged from 66% to 83%, which is suitable for the growth of the most edible fungi.

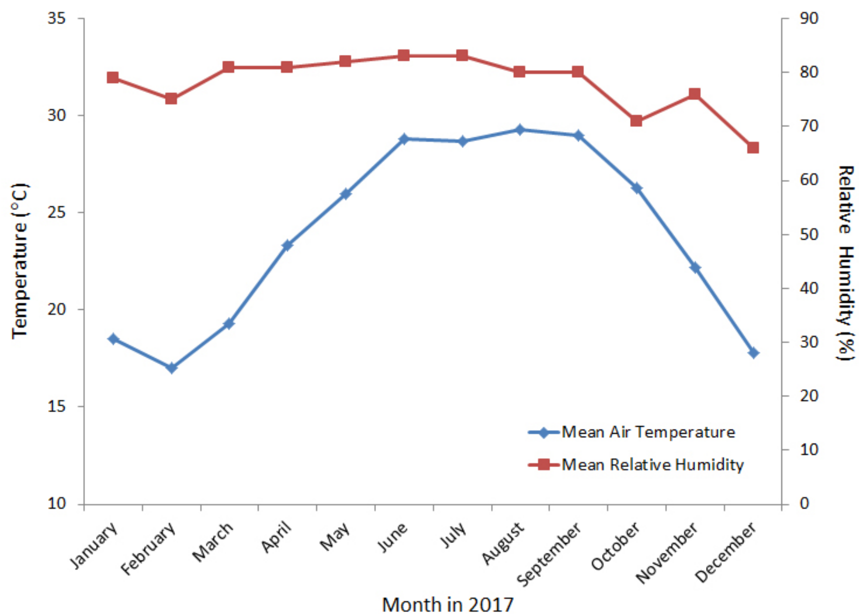

Fig. 1 Monthly mean daily air temperature variation and mean relative humidity in 2017.

Table 2. Temperature for fruiting body development of the edible fungus species cultivated in TMI.

	P. ostreatus				***P. geesteranus***	***G. lucidum***		***A. Aegerila***
Species	Suyin 6	H2	Huiping 03	Heiping 410	No.9	Hanzhi No.2	Chizhi G8	Chaxin AS1
Temperature (°C)	10-35	10-35	3-31	4-30	10-33	20-32	20-32	12-28

3. Cultivation of *Pleurotus ostreatus*

Four species Suyin 6, H2, Huiping 03 and Heiping 410 of *P. ostreatus* were cultivated in TMI by BDF formula in 2017. Suyin 6 and H2 were the summer species cultivated from May to October with the similar temperature for fruiting body development (10-35°C). Huiping 03 and Heiping 410 were the winter species cultivated in from November to April with temperature for fruiting body development of 4-30°C and 3-31°C respectively.

3.1 <u>Cultivation of winter species Huiping 03 and Heiping 410</u>

The color of the fruiting body of Heiping 410 is light grey; its pileus is big, with more bugs than other *Pleurotus ostreatus* species. While the color of fruiting body of Huiping 03 is deep grey; its pileus is tough, the yield is high with a shorter growth period. In order to confirm the optimal growth conditions for both winter species, the bioconversion rate were calculated and the results were plotted in Fig. 2. This figure shows that the bioconversion rates of both species is strongly correlated to the temperature of the month. February is the month with the lower mean daily air temperature in 2017, the highest bioconversion rates were obtained for both species in this year. The bioconversion rat for Heiping 410 is slightly higher than that of Huiping 03 in January, February and December suggested that Heiping 410 is more suitable to cultivate when the temperature is below 18°C, while Huiping 03 is more suitable to cultivate when the temperature is in between 18°C to 22°C, because higher bioconversion rate can be obtained in March and April when compares to that of Heiping 410.

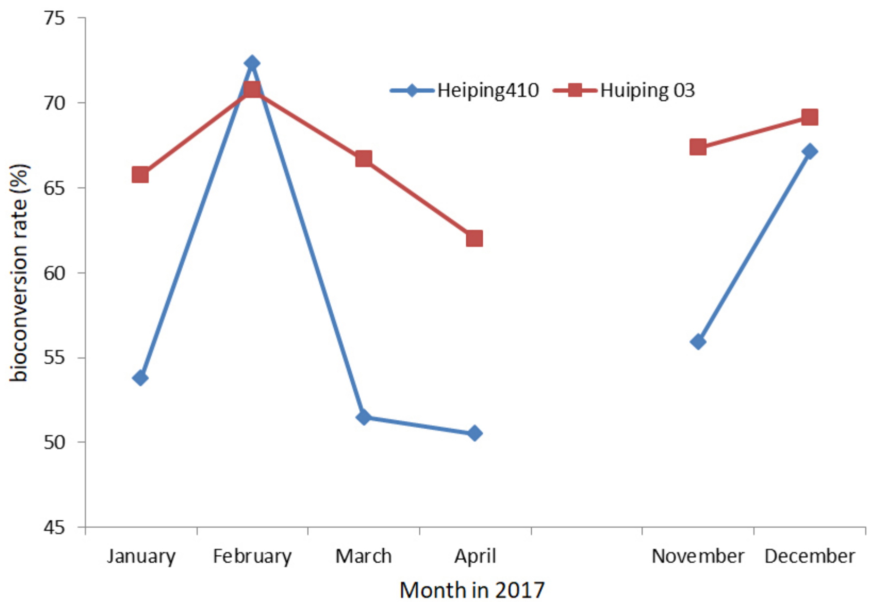

Fig. 2. Bioconversion rates of Heiping 410 and Huiping 03.

3.2 Cultivation of summer species Suyin 6 and H2

The color of the fruiting body of Suyin 6 is white; its pileus is big, with high growth and productive rate, while H3 is more resistance to high temperature. In order to confirm the optimal growth conditions for both summer species, the bioconversion rates of both species were calculated and the results were plotted in Fig. 3. This figure shows that the bioconversion rates of both species strongly correlated to the temperature of the month. The mean daily air temperatures of May and October were 26°C and 26.3°C respectively, which were the lowest temperatures in the growing season; the bioconversion rates both species gave the highest values in these two months. However when the maximum daily air temperature was over 30°C from June to September, the bioconversion rates of both species decrease sharply. When we compare the winter species with summer species, we find that the bioconversion rates of the summer species were much lower than those of the winter species, with only the yields in May and October are comparable to those of the winter species. It shows that although the two summer species can develop fruiting bodies in higher temperatures, but the optimal temperatures for the species are similar to those of the winter species (18-25°C), it is reasonable for most *P. ostreatus* that requires a mild temperature for development of fruiting body. It can be concluded that cultivation of *P. ostreatus* is not suitable in hot season in Hong Kong.

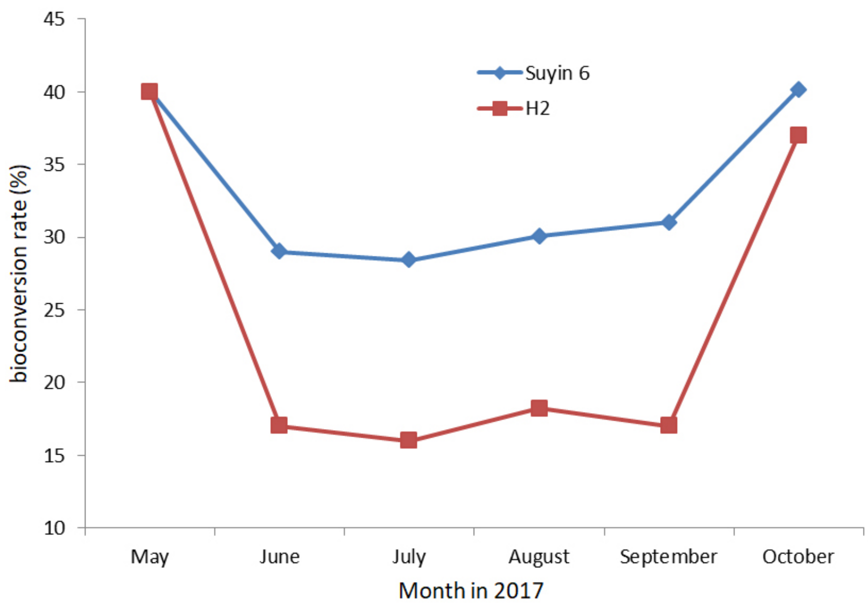

Fig. 3. Bioconversion rates of Suyin6 and H2.

4. Cultivation of *Pleurotus geesteranus* No.9

The shape of the fruiting body of *P. geesteranus* No.9 is round, taste is good and the growth period is short. In order to confirm the optimal growth condition for No. 9, the bioconversion rate was calculated and the result was plotted in Fig. 4. The bioconversion rate was higher from May to October. The mean daily air temperatures in these 6 months are in-between 24-27°C, and the mean daily maximum air temperatures are in-between 26-32°C. We can conclude that the air temperature affected the bioconversion rate of No.9 insignificantly, while a higher bioconversion rate obtained under a higher temperature. No.9 is suitable to cultivate in all months of a year.

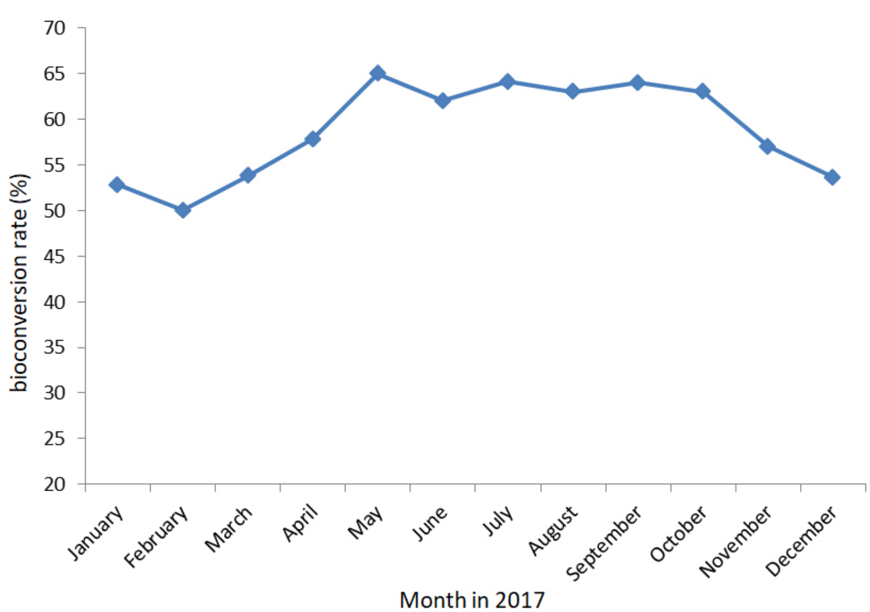

Fig. 4. Bioconversion rates of No.9.

5. Cultivation of *Ganoderma lucidum*

The cultivation of *G. lucidum* Meida BG and Tianzhi SG were carried out from May to August in 2017. The pileus of the fruiting body of Meida BG is big, with high growth rate and productive rate, while Tianzhi SG is more adaptable to the variation of growth conditions. In order to confirm the optimal growth conditions for both species, the bioconversion rates of both species were calculated and the results were plotted in Fig. 5. The figure shows that the bioconversion rate of Meida BG was higher than that of Tianzhi SG. And the influence of monthly mean daily air temperature variation to the yield is insignificant.

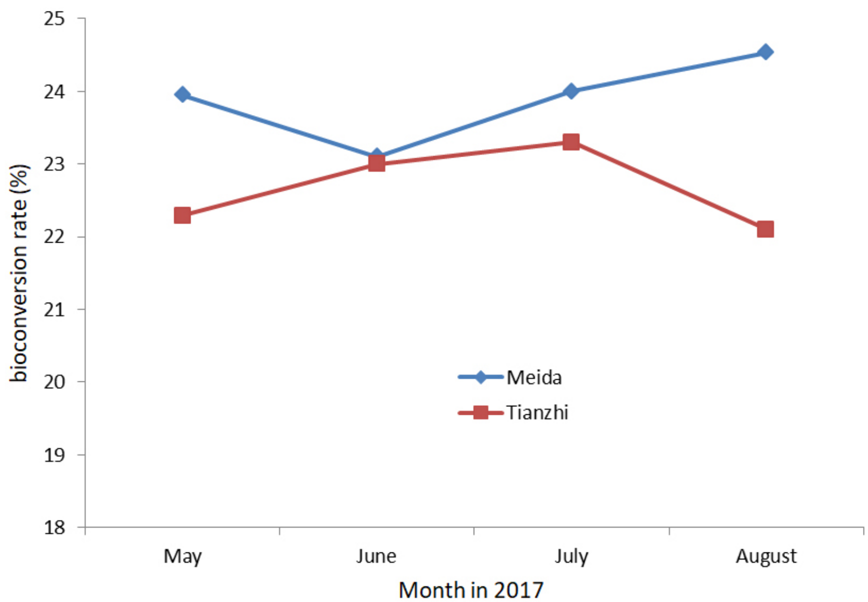

Fig. 5. Bioconversion rates of Meida and Tianzhi.

6. Cultivation of *Agrocybe aegerila* Chaxin AS1

The taste of the fruiting body of *A. aegerila* Chaxin AS1 is good and the growth period is short. It is a new species cultivated in March of 2017. The number of days for the first harvest, the maximum yield harvest and the last harvest were 24, 26 and 30 respectively. The yield per bottle was 38.05 g and 7.61 kg was obtained from 200 bottles. The bioconversion rate was 55.12%. The optimal temperature for cultivation is 20°C. It is a good candidate for cultivation in winter of the coming year.

7. Suggestions and discussion of organic non-environmental controlled cultivation of edible fungi

The bioconversion rates of all edible fungi cultivated in TMI are lower than those of commercialized cultivation. It may be related to: (1) the formulas in cultivation were not the most optimal; (2) the glass bottle used was not the suitable container for cultivation when compare to use plastic bag because the glass bottle can only provide one air contacting surface for fruit body growth while the plastic bag can provide more surface by cutting on it and therefore more fruit body can grow; (3) the volume for mycelium growth was small when using glass bottle, it can only provide enough nutrient for single harvesting; (4) lack of environmental control. To solve the problems we will arrange further experiments on: (1) formulas optimization; (2) design a suitable reusable glass container for cultivation; (3) optimization of the size of the container for multiple harvesting in order to increase the bioconversion rate and yield; (4) cooperate with the community to find the areas such as shopping malls and offices who have temperature control or have temperature regulation throughout the year for cultivation.

Conclusion

The eco-friendly mushroom cultivation of edible fungi in Hong Kong is feasible, but we should select suitable species to adapt the weather change under uncontrolled conditions. *P. ostreatus* and *A. aegerila* are the suitable species cultivated in cold season, while *G. lucidum* is the suitable species cultivated in hot season. *P. geesteranus* is the most suitable species cultivated in all month over the year. The bioconversion rates for different mushrooms cultivated in TMI are lower than those of commercialized cultivation because lack of environmental control, however our cultivation method utilizes the wastes from community can be shown as a demonstration of alternative environmental friendly method to the public for education.