INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
19F–158/202x/00–0–000–000
DOI: 10.17957/IJAB/15.1269
http://www.fspublishers.org

Full Length Article

Growth Conditions of *Termitomyces albuminosus* under Artificial Cultivation Conditions

Mingfu Gong[1,2*] and Qinlan Guan[1]
[1]*College of Life Sciences, Leshan Normal University, 614000, China*
[2]*Institute of Biodiversity Conservation and Utilization in Mount Emer, University of Leshan Normal, 614000, China*
[*]For correspondence: gongmingfu98@163.com

Abstract

Termitomyces albuminosus (Berk. Heim), or chicken mites, is a delicious edible fungus, but it is still difficult to artificially cultivate. In order to realize the artificial cultivation of chicken mites, a strain of *T. eurrhizus* was isolated from fruiting body. The optimum C, N sources, the concentration of KH_2PO_4 , $MgSO_4$, temperature and pH for mycelial growth were studied. The optimal culture medium for first-class spawn and second-class spawn of *T. eurrhizus* was screened. The results indicated that the optimal C, N sources for the growth of mycelia of *T. eurrhizus* were corn flour and yeast extract. The optimal KH_2PO_4 concentration for mycelium growth of *T. eurrhizus* was 0.15% (between 0.1 ~ 0.2%). The optimal $MgSO_4$ concentration for mycelium growth of *T. eurrhizus* was 0.050% (between 0.025 to 0.075%). The optimum temperature range for mycelium growth of *T. eurrhizus* was 26°C (between 22 ~ 30°C). The optimum pH range for mycelium growth of *T. eurrhizus* was between 4.5 to 8.0 and the optimum pH was 5.0. The formulation of optimal culture medium for first-class spawn of *T. eurrhizus* was glucose 20 g/L, potato 200 g/L, agar 20 g/L, yeast extract 2 g/L, VB1 3 mg/L, VB 66 mg/L. The formulation of optimum culture medium for second-class spawn of *T. eurrhizus* was 30% wood chips, 45% cotton seed shell, 22% bran, 1.5% sucrose, CaCO3 1%, 0.5% MgSO4, 70% termite nest leachate, water 70%. © 2020 Friends Science Publishers

Keywords: Chicken mites; *Termitomyces eurrhizus*; Culture medium; Growth rate; First-class spawn; Second-class spawn

Introduction

Termitomyces albuminosus (Berk. Heim), or chicken mites, belongs to Tricholomataceae, Agaricales, Hymenomycetes, Basidiomycotina (Heim, 1941; Zang, 1981; Wilson, 1995; Kirk *et al.*, 2001), also known as Chicken Mushroom, Umbrella Mushroom, Chicken Mites, Three Mushrooms, Triadella, Termite Mushroom, etc. (Fu and Li, 2009; Cai *et al.*, 2010), is a delicious fungus for food or medicine (Xiao *et al.*, 2014; Xu *et al.*, 2017b; Wei *et al.*, 2019). Chicken mites include the genus *Termitomyces* and the genus *Sinotermitomyces*. *Termitomyces* was proposed in 1942 for agaric symbiosis with termite (Baruah and baruwati, 2016). *Sinotermitomyces* is a new genus published by Mr. Zang by 1981 (Zang, 1981; Hu, 2001). At present, the artificial cultivation research of Chicken mites is still in the experimental stage (Li and Huang, 1995; Long and Zeng, 2007; Hu *et al.*, 2008). Because Chicken mites are symbiotic with termites, their fruiting bodies can only grow on termite nests (Li *et al.*, 2017). In addition, there are many kinds of microbes on the nests of termites, and fungi such as *Trichoderma*, *Penicillium*, *Xanthomonas* and yeast constitute a fungus "community" (Xu *et al.*, 2017a). Although chickens mites are the dominant fungus, the complex nutritional relationship and ecological background between chicken mites and termites have brought great difficulties to the artificial domestication of chicken mites. There are no reports of successful domestication and artificial cultivation of chicken mites in other countries, but there are more reports in China (Lai, 1993; Li and Huang, 1995; Yu *et al.*, 1997; Zhao *et al.*, 1998; Hu, 2001; Yao *et al.*, 2001; Fu *et al.*, 2013; Li *et al.*, 2017). But there have been no reports of successful artificial cultivation and mass production, indicating that there is still some difficulty in artificial cultivation of chicken mites (Yao *et al.*, 2001; Zhang *et al.*, 2010).

Chicken mites are chiefly distributed in South Africa, South Asia and some other subtropical regions. In China there are 24 species of *Termitemyces*, of which 20 species are distributed in Yunnan province, 9 species in Sichuan province, 8 species in Guizhou province, 4 species in Guangdong province, left only 1~2 species in other provinces (Hu *et al.*, 2008; Wang *et al.*, 2011; Zheng *et al.*, 2011; Tan, 2017). Chicken mites are not only fleshy fat, fine silky white, crisp and refreshing, delicious and rich in nutrients (Zou and Pan, 2009; Baruah and Baruwati, 2016), but also have the effects of clearing the spirit (Fang *et al.*, 2012; De *et al.*, 2018) enhancing immunity (Wang *et al.*, 2011; Hong and Ying, 2019; Wang *et al.*, 2019), curing phlegm (Qi *et al.*, 2000; Shi *et al.*, 2010), preventing

To cite this paper: Gong, M. and Q. Guan, 202x. Growth conditions of *Termitomyces albuminosus* under artificial cultivation conditions. *Intl. J. Agric. Biol.* 00: 000–000

intestinal cancer (Qu *et al.*, 2012; Zhang *et al.*, 2017), nourishing blood (Mitra *et al.*, 2016), moistening (Zou *et al.*, 2011), spleen (Li *et al.*, 2018) and stomach (Zhao *et al.*, 2016) and other effects (Wei *et al.*, 2007; Luo, 2010; Splivallo *et al.*, 2011; Li *et al.*, 2016; Li, 2018; Liu *et al.*, 2019). Although there are some reports on the domestication and cultivation of chicken mites, its artificial cultivation and production is still very difficult. (Fang *et al.*, 2012). There are many reports on chicken mites, such as the relationship between chicken mites and termites (Zang, 1981), the isolation and purification (Fu *et al.*, 2013), the classification and identification (Zang, 1981; Zou *et al.*, 2009; Yang *et al.*, 2012; De *et al.*, 2018), domestication and cultivation (Yu *et al.*, 1997; Zhao *et al.*, 1998; Zeng *et al.*, 2012; Yuan *et al.*, 2018; Xu *et al.*, 2019), the deep submerged fermentation (Xue *et al.*, 2013; Yan *et al.*, 2013) and the enrichment of chicken mites, etc (Zeng *et al.*, 2012; Tan, 2017).

In this study, the wild fruiting body was collected from Jiajiang County, Sichuan, China and a strain of *T. eurrhizus* was isolated from it. The optimum C, N source; concentrations of KH_2PO_4, $MgSO_4$; temperature and pH for mycelial growth were studied and the optimal culture medium for first-class spawn and second-class spawn of *T. eurrhizus* was screened.

Materials and Methods

Sample Collection and Morphological Identification

The fruiting body of chicken mites was collected from Jiajiang County, Sichuan Province, China on May 15, 2016. According to the morphological characteristics and molecular biological characteristics, the samples were identification by Edible Fungi Research Institute of Sichuan Province, China.

Culture Medium

The formulation of basal PDA comprehensive medium as showed in Table 1.

Culture mediums for first-class spawn of chicken mites have four formula. The formulation of PDA medium (a), modified PDA medium (b), PDA-rich medium (c) and nest leachate medium (d) are listed in Table 2.

Culture mediums for second-class spawn of chicken mites have three formula. The formulation of A, B and C medium are listed in Table 3.

Separation and Purification of *Termitomyces eurrhizus*

Impurities was removed from the base of the stipe. The surface of the fruit body was washed with sterile water and then surface disinfected with 75% ethanol solution. A soybean-sized tissue block at the intersection of the stipe and the cap was transferred to a PDA plate, and then placed at a temperature below 25°C, until the white hyphae grew.

Table 1: The formulation of basal PDA comprehensive medium

Component	Concentration
Potato	200 g/L
Glucose	20 g/L
Kh_2po_4	1 g/L
$Mgso_4$	0.5 g/L
Nacl	0.2 g/L
$Mnso_4$	0.2 g/L
Peptone	2.1 g/L
Vitamin B6	0.15 g/L

Note: The modified medium of *T. eurrhizus* was additionally increased by 0.075 g/L Vitamin B1 in the basal medium

Table 2: Culture mediums for first-class spawn of chicken mites

Formula names	Components
(a)	Glucose 20 g/L, Potato 200 g/L, Agar 20 g/L, and pH natural
(b)	Glucose 20 g/L, Potato 200 g/L, Agar 20 g/L, K2HPO4 1 g/L, KH2PO4 0.5 g/L, MgSO4•7H2O 0.1 g/L, tartaric acid ammonium 0.5 g/L, and trace element 1 mL to PDA medium
(c)	Glucose 20 g/L, Potato 200 g/L, Agar 20 g/L,2 g/L yeast extract, 30 mg/L vitamin B1and 60 mg/L vitamin B6
(d)	Glucose 20 g/L, Potato 200 g/L, Agar 20 g/L,160 mL termite nest leachate

Table 3: Culture mediums for second-class spawn of chicken mites

Formula names	Components
(a)	wood chips 30%, cotton seed shell 45%, bran 22%, sucrose 1.5%, CaCO3 1%, MgSO4 0.5%, water 140%
(b)	wood chips 30%, cottonseed shell 45%, bran 22%, sucrose 1.5%, CaCO3 1%, MgSO4 0.5%, 70% termite nest leachate, 70% water
(c)	wood chips 78%, bran 20%, Sucrose 1.0%, gypsum 1.0%, ant termite nest leachate 70%, water 70%

A few mycelium were picked and purified on the PDA slant medium until the hyphae were covered with a bevel. Afterwards, transferd to a 4°C refrigerator for storage.

Screening of Optimum Carbon and Nitrogen Sources for Mycelial Growth

The optimum carbon sources for growth of mycelial of *T. eurrhizus* was screened by the method that hyphae were inoculated into the basal medium with maltose, corn flour, ethanol, soluble starch, glucose, lactose and sucrose as carbon sources. The optimum nitrogen sources for growth of mycelial of *T. eurrhizus* was screened by the method that hyphae were inoculated into the basal medium with yeast extract, acid hydrolyzed casein, ammonium nitrate, bovine powder, soy flour, peptone, urea and as nitrogen sources. Three repetitions were set for each treatment, and all treatments were cultured under natural conditions of natural pH and about 25°C. The growth length of the hyphae was determined regularly every day.

Screening of Optimum Concentration of KH_2PO_4 and $MgSO_4$ for Mycelial Growth

The optimum concentration of KH_2PO_4 for growth of mycelial of *T. eurrhizus* was screened by the method that hyphae was inoculated into basal medium with 0.05%, 0.10%, 0.15%, 0.20%, 0.25% and 0.30% KH_2PO_4, respectively. The optimum concentration of $MgSO_4$ for growth of mycelial of *T. eurrhizus* was screened by the method that hyphae was inoculated into basal medium with 0.025%, 0.050%, 0.075%, 0.100%, 0.125%, and 0.150% $MgSO_4$, respectively. Three repetitions were set for each treatment, and all treatments were cultured under natural conditions of natural pH and about 25°C. The growth length of the hyphae was determined regularly every day.

Optimum Temperature and pH for Mycelial Growth

The optimum temperature for growth of mycelial of *T. eurrhizus* was screened by the method that hyphae was inoculated into basal medium and respectively cultivated at 22, 23, 24, 25, 26, 27, 28°C. The optimum pH for growth of mycelial of *T. eurrhizus* was screened by the method that hyphae was inoculated into basal medium with a pH of 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and cultivated at 25°C. Three repetitions were set for each treatment, and all treatments were cultured under natural conditions of natural pH and about 25°C. The growth length of the hyphae was determined regularly every day. The growth rate of the hyphae ($mm•d^{-1}$) was ratio of net growth of mycelium to growing days.

Screening of Culture Medium for First-Class Spawn of Chicken Mites

After the expansion, the strong hyphae were inoculated into four kinds of culture medium for first-class spawn of chicken mites, and cultured at 25°C. Observed from the second day after inoculation to record the density of the hyphae, the color of the hyphae and the growth of mycelium, calculate the mycelial growth rate.

Screening of Culture Medium for Second-Class Spawn of Chicken Mites

The strong hyphae were inoculated into three kinds of culture medium for second-class spawn of chicken mites, and cultured at 25°C. Observed from the second day after inoculation to record growth potential of the hyphae, infection fate, growth rate of hyphae, average full bottle days and the days from full bottle to mushroom.

Results

Identification of Chicken Mites

The cap was light white, and cap near the stipe was white, gray or yellowish. The stipe was nearly cylindrical. The cap was 6.5-8 cm and had obvious cusps. According to the morphological characteristics and the molecular biological characteristics, the sample was identified as *T. eurrhizus*.

Table 4: Effect of different carbon souce on the mycedium growth rate

Carbon Source	Mycelium growth rate($mm.d^{-1}$)				
	1	2	3	average	
Glucose	1.04	1.15	0.99	1.06	bB
Sucrose	1.09	1.16	1.14	1.13	bB
Maltose	0.95	0.99	1.15	1.03	bBC
Llactose	0.48	0.61	0.5	0.53	dE
Ethanol	0.79	0.82	0.81	0.81	cD
Corn flour	1.43	1.42	1.39	1.41	aA
Soluble starch	0.92	0.89	0.88	0.90	cCD

Note: Different lowercase letters in the same column means a difference in the 5% significance level. Different uppercase letters in the same column means a difference in the 1% significant level

Screening of Carbon Source for Mycelial Growth

As shown in Table 4, the mycelium of *T. eurrhizus* can grow with a variety of carbon sources and different carbon sources had different effects on mycelial growth (Table 4). Corn flour was the most suitable carbon source for mycelial growth of *T. eurrhizus*. When corn flour was used as the carbon source, the mycelial growth rate reached the maximum, which was 1.41 $mm•d^{-1}$. Other carbon sources were sorted by glucose, sucrose and maltose according to the influence of mycelial growth rate.

Screening of Nitrogen Source for Mycelial Growth

As shown in Table 5, the mycelium of *T. eurrhizus* can grow with a variety of niytohrn sources and different nitrogen sources had different effects on mycelial growth (Table 5). Yeast extract was the most suitable nitrogen source for mycelial growth of *T. eurrhizus*. When yeast extract was used as the nitrogen source, the mycelial growth rate reached the maximum, which was 1.49 $mm•d^{-1}$. Acid hydrolysis casein and ammonium nitrate had a great influence on mycelial growth of *T. eurrhizus*. The mycelial growth rate was 1.22 $mm•d^{-1}$ with acid hydrolysis casein and ammonium nitrate as nitrogen source, but significantly lower than mycelial growth rate with yeast as nitrogen source (Table 5).

Optimum Concentration of KH_2PO_4 and $MgSO_4$ for Mycelial Growth

As shown in Fig. 1, the concentration of KH_2PO_4 had a great influence on the growth of mycelium of *T. eurrhizus* (Fig. 1). The optimum KH_2PO_4 concentration rang for mycelial growth of *T. eurrhizus* were from 0.1% to 0.2%, and the mycelial growth rate, 1.10 $mm•d^{-1}$ was the fastest at 0.15% KH_2PO_4. The growth rate was was very slow below 0.1% KH_2PO_4 or above 0.2% KH_2PO_4. When the concentration of KH_2PO_4 was less than 0.15%, the growth rate of mycelium of *T. eurrhizus* was increased with the increase of KH_2PO_4 concentration. When the concentration of KH_2PO_4 was above 0.15%, the growth rate of mycelium of *T. eurrhizus* was decreased with the increase of concentration of KH_2PO_4 (Fig. 1).

Table 5: Effect of different nitrogen souce on the mycedium growth

Nitrogen Souce	Mycelium growth rate(mm.d-1)				
	1	2	3	average value	
Urea	0.82	0.78	0.84	0.81	dD
Ammonium nitrate	1.22	1.25	1.2	1.22	bB
Acid hydrolyzed casein	1.23	1.22	1.21	1.22	bB
Soy flour	0.82	0.78	0.81	0.80	dD
Beef extract	1.05	1.04	0.98	1.02	cC
Yeast extract	1.45	1.52	1.51	1.49	aA
Peptone	1.02	1.03	1.06	1.04	cC

Note: Different lowercase letters in the same column means a difference in the 5% significance level. Different uppercase letters in the same column means a difference in the 1% significant level

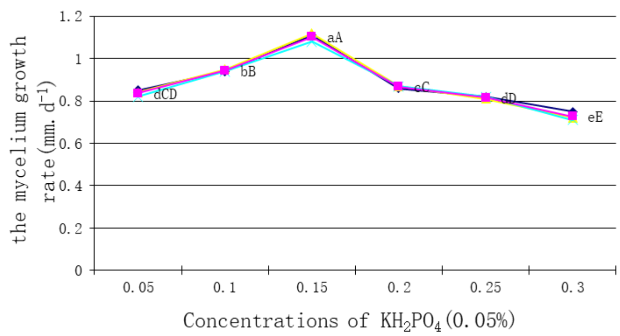

Fig. 1: Effect of different Concentrations of KH_2PO_4 on the mycelium growth rate

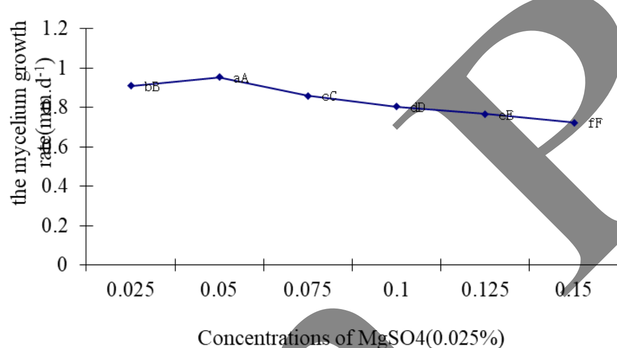

Fig. 2: Effect of different Concentrations of $MgSO_4$ on the mycelium growth rate

As shown in Fig. 2, the concentration of $MgSO_4$ had a great influence on the growth of mycelium of *T. eurrhizus* (Fig. 2). The optimum concentration of $MgSO_4$ for mycelial growth of *T. eurrhizus* was 0.05% and the preferred concentration range was from 0.025% to 0.075%. When the concentration of $MgSO_4$ was less than 0.05%, the growth rate of mycelium was increased with the increase of concentration of $MgSO_4$. When the concentration of $MgSO_4$ was higher than 0.05%, the growth rate of mycelium was decreased with the increase of concentration of $MgSO_4$ (Fig. 2.).

Optimum Culture Temperature for Mycelial Growth

As shown in Fig. 3, temperature had a great influence on the growth of mycelium of *T. eurrhizus* (Fig. 3). The optimum temperature for mycelial growth of *T. eurrhizus* was 26ºC

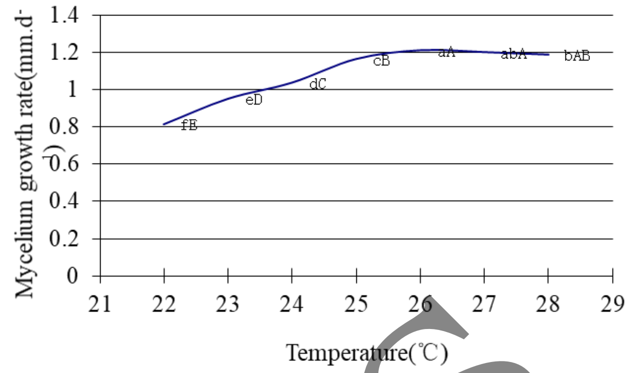

Fig. 3: Effect of different temperature(°C) on the mycelium growth rate

and the preferred temperature range was 26ºC degrees to 28ºC. When the temperature was lower than 26ºC, the mycelial growth rate was increased with the increase of temperature. When the temperature was between 26ºC and 28ºC, the mycelial growth rate was to be stable. (Fig. 3).

Optimum pH for Mycelial Growth

As shown in Fig. 4, temperature had a great influence on the growth of mycelium of *T. eurrhizus* (Fig. 4). The optimum pH for mycelial growth of *T. eurrhizus* was 5 and the preferred pH range was 4.5 to 6. When the pH was lower than 5, the mycelial growth rate was increased with the increase of pH. When the pH was higher than 5, the mycelial growth rate was decreased with the increase of pH. When the pH was between 6.5 to 8, the mycelial growth rate was to be stable. (Fig. 4).

Screening of Culture Medium for First-Class Spawn of Chicken Mites

The growing hyphae of *T. eurrhizus* were inoculated into four mediums for first-class spawn of chicken mites and the mycelial growth was obvious after the third day. All hyphae grew over the plate in about one week. According to the recorded results, the degree of mycelial density in the four mediums was shown as medium d=c>a>b. Mycorrhizal growth of mycelial was shown as the most abundant mycorrhizal in medium c and d, followed by a and the worst in b. The color of hyphae was shown as the most white in medium c, followed by d, poor in medium a, b. The growth rate of mycelium was shown as medium d=c>a>b. From the above analysis, it can be seen that the enriched medium c supplemented with yeast extract and vitamin is more conducive to the mycelial growth of chicken mites than the common medium, indicating that the yeast extract and vitamins are beneficial to the growth of the hyphae of the chicken mites (Table 6).

Table 6: Mycelial growth of the first-class spawn of chicken mites

Medium	Hyphae density	rhizomorph	Aerial hyphae	Hyphae color	Hyphae thickness	Mycelial growth rate (cm.d^{-1})
a	+	No	No	Light white	fine	1.75dD
b	++	Forming	Have	White	Slender	2.37bB
c	++	Forming	Developed	Thick white	Crude	3.36cC
d	+++	Forming	Developed	Thick white	sturdy	3.86aA

Note: Different lowercase letters in the same column means a difference in the 5% significance level. Different uppercase letters in the same column means a difference in the 1% significant level

Table 7: Mycelial growth of the second-class spawn of chicken mites

Medium	Germination rate(%)	growth rate(cm.d^{-1})	growth potential	Infection rate (%)	full bottle days (d)	mushroom days(d)
A	100	2.45bB	+++	5	65	25
B	100	2.68aA	+++	8	59	23
C	100	1.73cC	++	6	90	26

Note: Different lowercase letters in the same column means a difference in the 5% significance level. Different uppercase letters in the same column means a difference in the 1% significant level

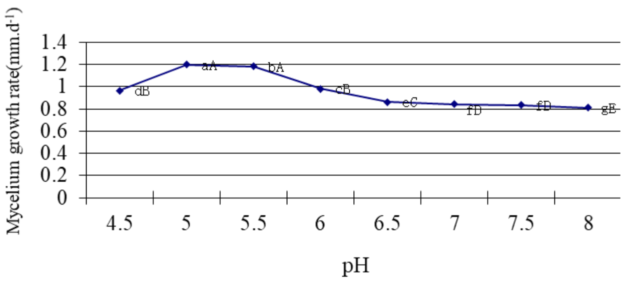

Fig. 4: Effect of different pH on the mycelium growth rate

Screening of Culture Medium for Second-Class Spawn of Chicken Mites

The growing mycelium of *T. eurrhizus* was inoculated into the three culture media for second-class spawn of chicken mites, and the flask was cultured in a constant temperature culture at 25°C and the germinations mion began on the second day. On the fifth day, the growth of hyphae was observed remarkably, and the hyphae were full of bottles in about fifth days. The results showed that the hyphae could grow in all three mediums, but the growth rate, hyphal color and growth potential in the medium A and B with cotton seed shells were all good, but the growth rate in medium B was higher than that in the medium A. Infection fate was shown as the medium B>C>A. It indicated that the formula with the nutrient solution was more infected. The hyphae in the medium C which uses bran instead of the cotton husks grew but did not grow well and was highly susceptible to infect (Table 7).

Discussion

The growth rate of the mycelium of *T. eurrhizus* was affected by the nutrient components such as carbon source and nitrogen source, culture conditions such as culture temperature and pH (Xiong *et al.*, 2011; Xiong and Li, 2013). In the medium containing the wasp's nest, the growth rate of hyphae of *T. tuliginous* was the fastest and the growth was better, and yeast extract, vitamins and other substances had a significant promoting effect on the growth of *T. tuliginous* (Fu *et al.*, 2013). Adding yeast extract 2 g/L, VB1 30 mg/L, VB6 60 mg/L and termite nest leachate to the first-class spawn medium had the effect of promoting growth of mycelium of *T. eurrhizus*. Under liquid culture conditions, the best formula for obtaining the highest mycelium biomass of *T. tuliginous* was bran 14g/L, corn flour 11 g/L, soy flour 2 g/L, KH_2PO_4 1.2 g/L, $MgSO_4$ 0.9 g /L (Xue *et al.*, 2013). The most suitable C/N for growth of the mycelium of *T. eurrhizus* was (12-14):1 when glucose was the carbon source and the amino acid was the nitrogen source, and the mycelium biomass was the highest when the required amount of VB1, VB2, VB3, VB5, VC and VM was 3.5 mg/L and when the requirement of VB6 and VB12 was 2.0-2.5 mg/L (Hu *et al.*, 2008).

The results of this experiment showed that the best carbon source for the growth of mycelium of *T. eurrhizus* was corn flour. This result was consistent with Li's findings (Li *et al.*, 2017), which was different from Yuan *et al.* (2018). The results of this experiment indicated that the best nitrogen source for the growth of mycelium of *T. eurrhizus* was yeast extract, followed by acid hydrolysis casein and ammonium nitrate, which was consistent with Yuan's finding (Yuan *et al.*, 2018) and was different from Li *et al.* (2017). Corn bran medium was more suitable for the growth of mycelium of *T. eurrhizus*. The mycelium yield was higher when the amount of corn flour and bran was 20 g/L and 10 g/L, respectively. In addition, the mycelial yield was affected by the experimental conditions on the basis of the culture medium such as the pH, temperature and rotation speed, and higher mycelial yield can be obtained under the conditions at pH 4.5, temperature 27°C, rotation speed 90 r/min. (Luo, 2010).

The results of research and experiments indicated that the chicken mites can indeed be artificially cultivated. The artificially cultivated chicken mites had the same external form and diffence flavor with wild chicken mites. The cultivated chicken mites was cultivated for too long times, the whole cultivation process taken more than half a year, only one oyster mushrooming period and low yield, and there was no commercial value. Perhaps it was for this reason that chicken mites were not artificially cultivated on a large scale. The author believes that if these two problems can be solved, the time for large-scale artificial cultivation of chicken mites will not be far behind.

Conclusion

The growth rate of the mycelium of *T. eurrhizus* was affected by the nutrient components such as carbon source, nitrogen source, KH_2PO_4 concentration and $MgSO_4$ concentration, culture conditions such as culture temperature and pH. The optimum carbon source, nitrogen source, KH_2PO_4 concentration and $MgSO_4$ concentration for mycelial growth of *T. eurrhizus* were corn flour, yeast extract, 0.15% and 0.05%, respectively. The optimum temperature and pH for mycelial growth are 26ºC and pH 5, respectively.

The formulation of optimal culture medium for first-class spawn of chicken mites was potato 200 g/L, glucose 20 g/L, agar 20 g/L, yeast extract 2 g/L, VB1 3 mg/L, VB6 6 mg/L. Among them, yeast extract 2 g/L, VB1 3 mg/L, and VB6 6 mg/L had a very significant promoting effect on the growth of mycelia mycelium of chicken mites, and organic matter, trace elements and other substances have little effect on the growth of mycelium. Termites nest leachate promoted the growth rate of hyphae, but its effect was not significant.

The formulation of optimum culture medium for second-class spawn of chicken mites was 30% wood chips, 45% cotton seed shell, 22% bran, 1.5% sucrose, 1% $CaCO_3$, 0.5% $MgSO_4$, 70% termite nest leachate, and water 70%.

Acknowledgement

We acknowledge the financial supports of the the scientific research fund of the Sichuan provincial education department (Grant NO: 15TD0026) and Key Research Project of Leshan Science and Technology Bureau (Grant NO: 13NZD103).

References

Baruah, N. and N. Baruwati, 2016. Awild edible mushroom *Termitomyces albuminosus* Berk and its importance among tribal people of Sivasagar district of Assam. *Intl. J. Sci.,* 11: 144–147

Cai, X.L., L.F. Yu and W. He, 2010. Advances in Studies on *Termitomyces* Heim Germplasm Resources. *J. Dali Univ.,* 9: 61–64

De, S.R.A., N.M. Kamat and V.S. Nadkarni, 2018. Purification and characterisation of a sulphur rich melanin from edible mushroom *Termitomyces albuminosus* Heim. *Mycology,* 9: 296–306

Fang, F., B. Xu, J.J. Li and Z.X. Huang, 2012. Transcriptome analysis of *Termitomyces albuminosus* reveals the biodegradation of lignocellulose. *Acta Microbiol. Sin.,* 52: 466–477

Fu, Q.F., X.D. Wang, J.Q. Wang, J. Yang, X.D. Wang, Y. Zhang and Y. Li, 2013. Separation and purification of black chicken bacterium and research on solid-liquid culture characteristics. *Edible Fungi,* 35: 18–19

Fu, Z.Y. and R.C. Li, 2009. Preliminary study on the relationship between genus and genus. *J. Fujian Agric. For. Univ.-Nat. Sci. Ver.,* 38: 271–274

Kirk, P.M., P.F. Cannon, J.C. David and J.A. Stalpers, 2001. *Ainsworth & Bisby 's Dictionary of the Fungi*. Wallingford, UK: CAB1 Publishing

Heim, R., 1941. Etudes descriptives et experimentales sur lesagarics termi-tophiles Afrique tropicale. *Mem. Del. Acad. Sc.,* 64: 25–29

Hong, Y.W. and T.J. Ying, 2019. Characterization of a chitin-glucan complex from the fruiting body of *Termitomyces albuminosus* (Berk.) Heim. *Intl. J. Biol. Macromol.,* 134: 131–138

Hu, Q.X., 2001. The present research situation of *Termitomyces albuminosus*. *Acta Edulis Fungi,* 8: 54–58

Hu, S.Q., T.G. Liu and X.B. Li, 2008. Research of the Nutrition Requirement of the *Termitomyces albuminosus*. *J. Food Sci. Biotechnol.,* 27: 67–70

Lai, J.P., 1993. Domestication and high-yield cultivation of *Termitomyces albuminosus*. *Chin. Edible Fungi,* 12: 24–26

Li, L. and Z.G. Huang, 1995. Domesticated cultivation experiment of chicken mites. *Edible Fungi,* 6: 10

Li, M.H., G.H. Li, S.H. Pu and F.S. Cui, 2018. Study on the Composition and Antioxidant Activities of Different Solvent Extracts of *Termitomyces albuminosus*. *Food Machin.,* 34: 144–148

Li, X.L., J. Liu, Y.T. Yang, Y.Y. Hu, B.D. Liang and S.J. Li, 2016. Effects of 1-MCP on postharvest physiology and storage quality of *Termitomyces albuminosus*. *Food Sci.,* 37: 237–241

Li, Y., 2018. HPLC Determination of Water-Soluble Vitamins in *Termitomyces albuminosus*. *Food Res. Develop.,* 39: 124–128

Li, Y.L., B.W. Den, M.M. Huang, X.C. Xie and H. Peng, 2017. Isolation and growth conditions of *Termitomyces albuminosus*. *Heilongjiang Agric. Sci.,* 10: 87–91

Liu, J., X.L. Li, H.X. Wei, M. Zhao, L.P. Xue and X.P. Wang, 2019. Optimization of Ultrasound-assisted Extraction of Crude Protein from *Termitomyces albuminosus* and Its Antioxidant Activity. *Sci. Technol. Food Indus.,* 40: 221–226

Long, Z.H. and D.X. Zeng, 2007. Preliminary study on the culture characteristics of *Termitomyces albuminosus*. *Food and Biotechnol.,* 26: 90–93

Luo, X.M., 2010. Optimization of liquid-state fermentation conditions for mycelium of *Termitusuomyces albuminos*. *Food Sci. Technol.,* 35: 29–33

Mitra, P., N.C. Mandal, K. Acharya and J. Verbr, 2016. Polyphenolic extract of *Termitomyces heimii*: antioxidant activity and phytochemical constituents. *J. Verbr. Lebensm.,* 11: 25–31

Qi, J.H., M. Ojika and Y. Sakagami, 2000. Termitomycesphins A–D, Novel Neuritogenic Cerebrosides from the Edible Chinese Mushroom *Termitomyces albuminosus*. *Tetrahedron,* 56: 5835–5841

Qu, Y., K.Y. Sun, L.J. Gao, Y. Sakagami, H. Kawagishi, M. Ojika and J.H. Qi, 2012. Termitomycesphins G and H, additional cerebrosides from the edible Chinese mushroom *Termitomyces albuminosus*. *Biosci., Biotechnol. Biochem.,* 76: 791–793

Shi, Y.Z., Y.X. He, J. Long and Z. Li, 2010. Study on the biophysical characteristics and pharmacodynamics of *Termitomyces albuminosus*. *Psychosomatic Doctor,* 6: 92–93

Splivallo, R., S. Ottonello, A. Mello and P. Karlovsky, 2011. Truffle volatiles: from chemical ecology to aroma biosynthesis. *New Phytologist,* 189: 688–699

Tan, J.D., 2017. Study on the germplasm resources of chicken sputum. *Shanxi Agric. Econ.,* 4: 50–52

Wang, S.L., H.M. Wang, Y.Y. Zhao, W.Y. Xiao and Z.Q. Huang, 2019. Effects of polysaccharides from *Termitomyces albuminosus* sputum on growth performance, antioxidant and immune function. *Freshwater Fish.,* 49: 87–91

Wang, S.L., K.Y. Wang and Y.L. Peng, 2011. Immuneacivity of apolysaccharide isolated from wild *Collybia albuminosa*. *Chin. Vet. Sci.,* 41: 1276–1281

Wei, X.M., S.Q. Hu, W. Wei and X.B. Li, 2007. Analysis of the synthesis of protein in mycelium of *Termitomyces albuminosus* under different conditions. *Food Sci. Technol.,* 32: 74–77

Wei, Y.S., Y.H. Hou, J.X. Li and M.Y. Zheng, 2019. Quantitative Analysis of Mineral Element Composition in Wild *Termitomyces albuminosus* by ICP-OES. *Food Indus.,* 40: 294–298

Wilson, D., 1995. Endophyte aterm, aclarification itsuse definition. *Oikos*, 73: 274–276

Xiao, Y., Z. Li and W. Shi, 2014. Determination of Nutrient Components and Heavy Metals in Different Parts of Fruits of *Termitomyces tuliginous*. *Chin. Brewing*, 33: 142–144

Xiong, Y. and M.J. Li, 2013. Optimization of the basic medium of mycelium of *Termitomyces albuminosus*. *Food Machin.*, 29: 185–189

Xiong, Y., M.J. Li and J. Zhou, 2011. Effects of pH and Temperature on the Growth of PXT-1 Strain of Panzhihua Wild *Termitomyces albuminosus*. *Northern Hortic.*, 11: 164–165

Xu, B., Y.Q. Zhang and H. Yan, 2017a. Optimization of Solid Medium of *Termitomyces albuminosus*. *Food Res. Develop.*, 38: 175–178+221

Xu, B., L.X. Zheng, W. Shi, Y.J. Zhang and H. Yan, 2017b. Study on the Optimization of Ultrasonic Extraction of Polysaccharide from *Termitomyces tuliginous*. *Food Res. Develop.*, 38: 38–40

Xu, N., L.G. Feng, C.H. Wang, Z.L. Deng, S.C. Zou and H. Lu, 2019. Optimization of culture conditions for fermenters of *Termitomyces tuliginous*. *S. Chin. J. Agric. Sci.*, 50: 344–349

Xue, L.H., X.D. Wang, H. Cai, H.C. Fan, H. Dang and Y. Li, 2013. Optimization of Liquid medium for *Termitomyces tuliginous*. *J. Neijiang Normal Univ.*, 28: 24–27

Yao, X.H., Y.X. Xu and S.C. Xu, 2001. Advances in research on biological characteristics and deep fermentation of *Termitomyces albuminosus*, *Acta Edulis Fungi*, 8: 59

Yan, Y., Z.M. Lu, Y.Y. Xu, J.S. Shi, Y.Y. Wand, G.H. Xu and Z.H. Xu, 2013. Analysis of the Composition of Liquid Fermentation of *Termitomyces albuminosus*. *J. Edible Fungi*, 20: 60–63

Yang, F., B. Xu, S.J. Zhao, J.J. Li, Y. Yang, X. Tang, F. Wang, M.Z. Peng and Z.X. Huang, 2012. De novo sequencing and analysis of the termite mushroom (*Termitomyces albuminosus*) transcriptome to discover putative genes involved in bioactive component biosynthesis. *J. Biosci. Bioengineer.*, 114: 228–231

Yu, C.X., X.K. Liu and S.G. Zhao, 1997. A brief report on the test of *Termitomyces albuminosus* in bottle. *Edible Fungi*, 19: 32–33

Yuan, Z.C., L.N. Xue, C.L. Su, Y.Y. Yang, Y. Jiang, W. Li and M.H. Mo, 2018. Optimization of cultural conditions of uncultured *Termitomyces albuminosus* and the screening of its nutrition ingredient. *Heilongjiang Agric. Sci.*, 11: 109–111

Zang, M., 1981. Notes on the classification and distribution of *Termitomyces* from Yunnan. *Acta Botanica Yunnanica*, 3: 367–374

Zeng, X.F., Y.T. Chen, W.Q. Xiong and X.B. Luo, 2012. Chicken sputum mycelium culture test. *Edible Fungi*, 34: 9–18

Zhang, H., Q.R. Zheng and M. Li, 2017. Study on ultrasonic-assisted extraction of polysaccharides from ch *Termitomyces tuliginous* and its antioxidant activity by response surface methodology. *Food Fermentation Technol.*, 53: 13–18+121

Zhang, Y.J., H.C. Guo and R.C. Li, 2010. Current status of domestication and cultivation of chicken mites. *Acta Microbiol. Sin.*, 50: 1288–1292

Zhao, H.J., S.S. Li, J.J. Zhang, G. Che, M. Zhou, M. Liu, C. Zhang, N. Xu, L. Lin, Y. Liu and L. Jia, 2016. The antihyperlipidemic activities of enzymatic and acidic intracellular polysaccharides by *Termitomyces albuminosus*. *Carbohydrate Polymers*, 151: 1227–1234

Zhao, S.G., X.K. Liu and M.D. Chen, 1998. Introduction and domestication cultivation of chicken mites. *Chin. Edible Fungi*, 17: 11–13

Zheng, S.Y., H.X. Wang and G.Q. Zhang, 2011. A novel alkaline protease from wild edible mushroom *Termitomyces albuminosus*. *Acta Biochim. Polonica*, 58: 269–273

Zou, L.K. and X. Pan, 2009. Total DNA isolation of *Termitomyces albuminosus* and cloning of its ITS region. *Northern Hortic.*, 6: 217–219

Zou, L.K., X. Pan, J.T. Han, M.F. Yang and B.X. Wei, 2009. The Morphology and Molecular Identification of *Termitomyces albuminosus*. *Edible Fungi*, 31: 17–18

Zou, L.K., X. Pan, A.L. Yue, Y. Luo, W. Li, Y. Zhang, Q. Yao, Q. Wu and L. Zheng, 2011. Analysis of Amino Acid Composition and Selenium Content of *Termitomyces albuminosus* in Sichuan Province. *Food Sci.*, 32: 245–248

(Received 12 September 2019; Accepted 20 September 2019)