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Mycelial Growth of *Paecilomyces hepiali* in Various Agar Media and Yield of Fruit Bodies in Rice Based Media

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ABSTRACT

Growth of *Paecilomyces hepiali* in various agar media and yield of fruit bodies in rice based media were studied. The best growth in agar media was obtained at 25˚C (61.86 mm colony diameter in 14 days). The initial agar media pH range from 6 to 8 was found to be the most favourable for mycelial growth. This study found that agars made with powders of cereal grains alone do not support good mycelial growth of *P. hepiali*. Addition of peptone improved mycelial growth significantly. The most favourable carbon sources were Mannose, Fructose and Glucose. Organic nitrogen sources were found to be the most preferred. The results demonstrated that brown rice is better than polished rice in yield of fruit bodies. Addition of peptone was found to be quite significant in enhancing yield of fruit bodies. Peptone, as a supplement, gave a better yield than addition of egg yolk, albumen and a mixture of the two. The medium with 40 g brown rice, 0.325 g glucose, 0.65 g sucrose, 2 g peptone and 65 ml corn steep liquor was found to be the most favourable and it yielded 19.3 g of fresh fruit bodies.

Keywords: Agar Media; Cereal Grains Agar; Mycelial Growth; *Paecilomyces hepiali*

1. Introduction

Paecilomyces hepiali is one of the most popular fungi isolated from natural *Ophiocordyceps sinensis*. Until recently, this fungus had been regarded as one of the true anamorphs of *O. sinensis*. Mycologists have reported the isolation of more than 10 fungal species from natural *O. sinensis*. The isolations have resulted in the commercialization of several mycelia fermentation products that are used to supplement the limited availability of wild *O. sinensis* [1]. For instance, JinShuiBao capsule, the commercial product of Cs-4 (*Paecilomyces hepiali*, a standardized mycelium of *O. sinensis*) has been used in clinics throughout China. *Synnematum sinensis*, *Cephalosporum sinensis*, *Gliocladium roseum*, and *Mortierella hepialid*, the fungus strains isolated from natural *O. sinensis*, have also been subjected to large-scale fermentation and are used as commercial products [2]. Most of the recent studies are reporting *Hirsutella sinensis* as a generally accepted anamorph of *O. sinensis* [3-6].

Ophiocordyceps sinensis, (Berk.) (G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora) is one of the most popular and highly valued medicinal fungi. In 2007 it was transferred to a new family Ophiocordycipitaceae and genus *Ophiocordyceps* from the previous family Clavicipitaceae and genus *Cordyceps* as described in the phylogenetic study conducted by Sung *et al.* [3]. Its fruit body extends from the mummified carcass of an insect larvae, the Himalayan moth, *Thitarodes armoricanus* (*Hepialis armoricanus*). In nature, it is found only at alpine pastures in north-west and central Himalayan region [7]. This fungus is called “Dong Chong Xia Cao” (Winter-Worm-Summer-Grass) in Chinese and “Tochukaso” in Japanese. In Nepal, Bhutan, and India, it is popularly known as “Yartsagumba”, “Yartsa Goenbub”, and “Keera Jhar”, respectively [2].

The mycelial powder of *P. hepiali* has been intensively studied and developed into functional food in China for many years. Polysaccharides, adenosine and cordycepin in the mycelial powder of *P. hepiali* are considered as the major functional compositions for the health effects [8]. Studies have shown that *P. hepiali* can inhibit tumor proliferation, invasion, metastasis, and neovascularisation, induce apoptosis, reverse drug resistance, enhance immunity, and protect hepatic function [9].

Pure cultures of various fungi have been isolated and are being maintained in various laboratories around the

World. These cultures are mostly maintained in a variety of agar media in petri dishes/plates or test tube slants. Continuous explorations on mycelial growth of various fungi in agar media are, therefore, still necessary. This ensures availability of many options of agar media recipes which are favourable for mycelial growth of a particular fungus. As Stamets [10] pointed out, a strain grown on one medium always adapts to it, and may lose its innate ability to digest larger, more complex and variable substrates.

Most of the dietary supplements and medicinal products from mushrooms are made only from artificially grown mycelium. Apart from the extra effort required to produce the fruit body, some mushrooms do not contain any better amounts of bioactive compounds in their fruit body than the mycelium. Therefore, it is not necessary to produce fruit bodies for such mushrooms. Holliday *et al.* [11] reported that formation of the fruit body in artificial cultivation of *O.* sinensis does not result in any significant change to the analytical chemistry profile found in its mycelium. However, New Chapter Inc. [12] indicates that the full health benefits of a medicinal mushroom can be obtained through a combination of all the stages of its life-cycle which include mycelium, fruit bodies, spores and their extracellular compounds. It is for this reason that we think it is still necessary to continue with the pursuit for better fruiting methods of *P. hepiali.*

2. Materials and Methods

2.1. Fungal Material

The fungal strain used in this study was obtained from the mushroom culture bank at the Laboratory of Forest Production Control, Kyushu University. It is assigned to accession number KUMB108 in the culture bank. The strain was originally brought from Jilin Agricultural University, China. After genetic analysis its internal transcribed spacer (ITS) sequence was found to be an exact match (100%) of NCBI Gene bank entry EF555097.3—*Paecilomyces hepiali* strain Ph-4Qinghai. In the NCBI Gene bank this strain had been recorded as *Ophiocordyceps sinensis* Ph-4Qinghai before being updated to *Paecilomyces hepiali* strain Ph-4Qinghai.

2.2. Growth in Agar Media

Effect of temperature and initial pH on mycelial growth

To determine the most favourable temperature for mycelial growth of *P. hepiali*, 5 mm diameter agar plugs with actively growing mycelium were inoculated on PDA plates by placing on the centre of the PDA surface. Five inoculated plates were then incubated at each of the following temperatures: 5˚C, 10˚C, 15˚C, 20˚C, 25˚C and 30˚C. On the 14th day after inoculation, colony diameter measurements were made. Just as in Imtiaj *et al.* [13], the diameter measurement recorded for each petri dish was an average of three diameter measurements on that particular dish.

To investigate the effect of initial pH on mycelial growth, PDA media with pH 5, pH 6, pH 7, pH 8 and pH 9 were prepared. Adjustments to various pH levels were made using 1N HCl and 1N NaOH before autoclaving the media for 15 minutes at 121˚C. Plates were inoculated as described earlier. Measurements of colony diameter were preformed on the 14th day of incubation at 25˚C.

Mycelial growth in various cereal grain agar media

Investigation on the effect of cereal grain agar media on mycelial growth of *P. hepiali* was conducted by growing in 90 mm petri dishes containing the following twelve agar media:

- Brown rice agar (BRA): 20.0 g brown rice powder, 15.0 g agar and 1000 ml distilled water;
- Brown rice peptone agar (BRPA): 20.0 g brown rice powder, 4.0 g peptone, 15.0 g agar and 1000 ml distilled water;
- Lye agar (LA): 20.0 g lye powder, 15.0 g agar and 1000 ml distilled water;
- Lye peptone agar (LPA): 20.0 g lye powder, 4.0 g peptone, 15.0 g agar and 1000 ml distilled water;
- Millet agar (MA): 20.0 g millet powder, 15.0 g agar and 1000 ml distilled water;
- Millet peptone agar (MPA): 20.0 g millet powder, 4.0 g peptone, 15.0 g agar and 1000 ml distilled water;
- Oats agar (OA): 20.0 g oats powder, 15.0 g agar and 1000 ml distilled water;
- Oats peptone agar (OPA): 20.0 g oats powder, 4.0 g peptone, 15.0 g agar and 1000 ml distilled water;
- Wheat agar (WA): 20.0 g wheat powder, 15.0 g agar and 1000 ml distilled water;
- Wheat peptone agar (WPA): 20.0 g wheat powder, 4.0 g peptone, 15.0 g agar and 1000 ml distilled water;
- White sorghum agar (WSA): 20.0 g white sorghum powder, 15.0 g agar and 1000 ml distilled water;
- White sorghum peptone agar (WSPA): 20.0 g white sorghum powder, 4.0 g peptone, 15.0 g agar and 1000 ml distilled water.

The media were sterilized for 20 minutes at 121˚C before pouring into petri dishes. Three petri dishes for each of the above agar media were inoculated, at the centre, with 5 mm diameter agar plugs containing actively growing mycelium. The petri dishes were then incubated at 25˚C in the dark. On the 15th day after inoculation, measurements of colony diameters were made as previously explained.

Effect of carbon and nitrogen sources on mycelial growth

Eleven carbon sources namely cellulose, fructose, ga-

lactose, glucose, lactose, maltose, mannitol, mannose, sorbitol, sucrose and xylose were tested. Modified mushroom complete medium (MMCM) was used for this experiment. The mushroom complete medium (20.0 g glucose, 2.0 g peptone, 2.0 g yeast extract, 1 g K_2HPO_4, 0.5 g $MgSO_4$, 0.5 g KH_2PO_4, 20.0 g agar and 1000 ml distilled water) in Shim *et al.* [14] was modified by removing 2 grams of yeast extract and raising the quantity of peptone from 2 to 4 grams to make MMCM. A medium for each carbon source was prepared by adding 20 g to the MMCM to replace glucose. A medium without carbon source served as a control. Diameter measurements of the colonies were done on the 14th day of incubation at 25˚C just the same way as mentioned above.

The effect of nitrogen sources (alanine, ammonium acetate, ammonium chloride, ammonium nitrate, ammonium phosphate—dibasic, ammonium phosphate—monobasic, ammonium sulphate, beef extract, calcium nitrate, glycine, arginine, L-histidine, L-methionine, peptone, potassium nitrate, urea and yeast extract) was assessed by replacing peptone in MMCM with 4 g of each of the nitrogen sources. A medium without nitrogen source served as control.

2.3. Growth in Rice-Based Media

Ten different rice-based media, as presented in **Table 1**, were prepared to investigate their effect on yield of fruit bodies. The method in Yang and Ohga [15] was used to prepare the substrates and cultivate the fungus to fruiting. The contents of each of the media, shown in **Table 1**, were put into a 300 ml Polypropylene Conical Flask, mixed well, and then left overnight in a refrigerator at 4˚C. This was done to allow the rice to absorb water and various nutrients in the mixture. The media were then autoclaved for 30 minutes at 121˚C.

After cooling, each medium was inoculated with three 4 mm diameter agar plugs with actively growing *P. hepiali* mycelium. The three plugs were placed around the centre of the medium surface. Each medium was replicated three times.

The media were then incubated at 23˚C in the dark. After full colonisation, the temperature was changed down to 17˚C for a week to induce fruiting. Following a week of incubation at 17˚C, the temperature and humidity in the growth chamber were set to 21˚C and 85% - 95% respectively. During this time, the media were exposed to 8 hours of fluorescent light every day. As the temperature was being reset to 21˚C, the silicone plugs which had been used to cover the openings of the Conical Flasks were removed.

2.4. Data Analysis

Data collected were subjected to Analysis of Variance (ANOVA) and Tukey's Multiple Comparison Test, at 5% level of significance, using Minitab 16 statistical software.

3. Results and Discussion

3.1. Effect of Temperature and Initial pH on Mycelial Growth

Temperature had a significant effect on mycelial growth of *P. hepiali* (**Table 2**). The highest growth was obtained at 25˚C. This is same as findings reported on other fungal species namely *Cordyceps nutans* Pat. [16], *Ophiocordyceps longissima* [17], *Ophiocordyceps heteropoda* [18], *Cordyceps cardinalis* [19] and *Cordyceps sinensis* (now known as *Ophiocordyceps sinensis*) [20]. It was interest-

Table 1. Rice-based media.

Media	Polished rice (g)	Brown rice (g)	Egg (g)	Glucose (g)	Sucrose (g)	Peptone (g)	Corn steep liquor (ml)
*A*1	40		20[*1]	0.325	0.65		65
*A*2		40	20[*1]	0.325	0.65		65
*B*1	40		20[*2]	0.325	0.65		65
*B*2		40	20[*2]	0.325	0.65		65
*C*1	40		20[*3]	0.325	0.65		65
*C*2		40	20[*3]	0.325	0.65		65
*D*1	40			0.325	0.65	2	65
*D*2		40		0.325	0.65	2	65
*E*1	40			0.325	0.65		65
*E*2		40		0.325	0.65		65

Yolk[*1] Albumen[*2] Yolk + Albumen[*3].

ing to note that even at 5˚C there was some growth at the rate of around 0.89 mm/day in terms of colony diameter.

As shown in **Table 2**, initial media pH values studied did not have much effect on mycelial growth. The highest colony growth was obtained at pH 7. This was similar to previous studies on other fungal species [16-18]. However, pH 7 was not statistically different from pH 6 and 8 ($p > 0.05$). Amin *et al.* [20] also reported that no significant variations were found in growth rate of *O. sinensis* mycelium growing in PDA Media with different initial pH.

3.2. Mycelial Growth in Various Cereal Grains Agar Media

In terms of thickness of the colonies, a remarkable difference was observed between the medium with and without peptone for each of the six studied grains (**Figure 1**). All the media with peptone produced thick and white mycelia. Millet agar (MA) and white sorghum agar (WSA) produced very thin and transparent colonies. The colony in brown rice agar (BRA) was thin and translucent towards the edge. Lye agar (LA) and oats agar (OA) produced mycelial mats which were white, thin and without well developed aerial mycelium. In wheat agar (WA) the colony was white and with well developed aerial mycelium just like in grains media with peptone. As shown in **Figure 2**, apart from in wheat and millet, addition of peptone did not have a significant effect in terms of colony diameters ($p > 0.05$) in the other four grains. The highest colony diameter (79.99 mm), in 15 days, was obtained in wheat peptone agar (WPA). However this was not significantly different from colony diameters in BRA (68.89 mm) and LA (68.44 mm). The lowest colony diameter was recorded in MA (56.75 mm).

The results show that inclusion of peptone to the cereal grains agars is necessary for best results in mycelial growth of *P. hepiali*. Apart from wheat, the other grains were not that favourable in supporting mycelial growth without addition of peptone. In their study on nutritional requirements of mycelial growth of *Cordyceps sinensis* in submerged culture, Dong and Yao [21] found the media supplied with peptone and beef extract to be the most effective for the mycelial growth of *C. sinensis*. They also indicated that this fungus had greater preference on organic nitrogen, which is common in fungi. This could also explain why in this study all the cereal grains agar media supplied with peptone were favourable in sup-

Table 2. Effect of temperature and initial pH on mycelial growth of *Paecilomyces hepiali* in PDA.

Temperature (Deg. Celsius)	Colony diameter (mm)	Initial pH	Colony diameter (mm)
5	12.53 ± 0.50^{f}	5.0	56.66 ± 3.02^{b}
10	28.59 ± 0.60^{e}	6.0	61.73 ± 1.86^{a}
15	36.13 ± 0.83^{d}	7.0	61.79 ± 1.50^{a}
20	55.80 ± 2.38^{b}	8.0	60.53 ± 1.43^{a}
25	61.86 ± 0.96^{a}	9.0	59.20 ± 1.70^{ab}
30	31.80 ± 0.93^{c}		

Values in the same column with different superscript letters differ significantly according to Tukey's Multiple Comparisons test ($p < 0.05$). Each value is a mean ± SD of five replicates measured on the 14th day after inoculation.

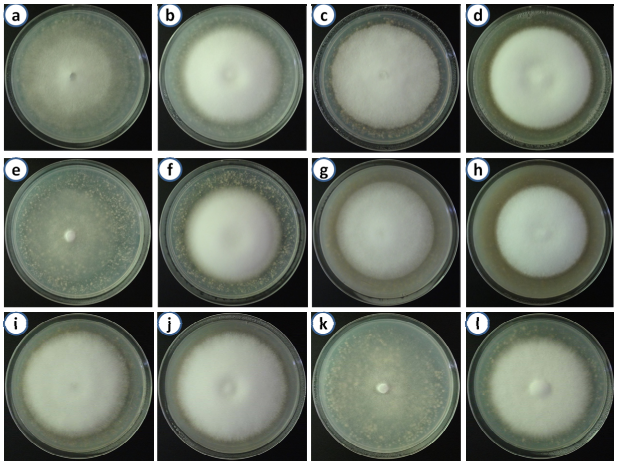

Figure 1. Colonies of *Paecilomyces hepiali* in different cereal grains agars on the 15th day after inoculation: a-brown rice agar (BRA); b-brown rice peptone agar (BRPA); c-lye agar (LA); d-lye peptone agar (LPA); e-millet agar (MA); f-millet peptone agar (MPA); g-oats agar (OA); h-oats peptone agar (OPA); i-wheat agar (WA); j-wheat peptone agar (WPA); k-white sorghum agar (WSA); l-white sorghum peptone agar (WSPA).

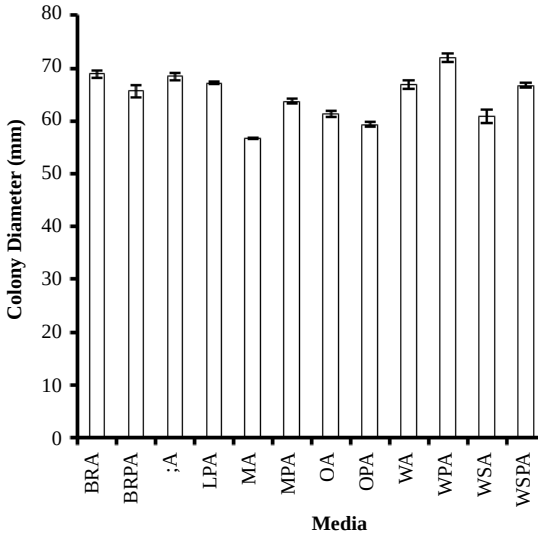

Figure 2. Mycelial growth of *Paecilomyces hepiali* in various cereal grains agar media. Each bar represents the mean value of three replicates measured on the 15th day after inoculation. The error bars represent standard error.

porting mycelial growth of *P. hepiali*.

3.3. Effect of Carbon Source on Mycelial Growth

The growth response of *P. hepiali* to various carbon sources investigated was significant (**Figures 3** and **4**). The most favourable carbon sources were mannose, fructose and glucose with colony diameter values of 68.20 mm, 67.73 mm and 67.13 mm respectively, in 14 days. These values were not statistically different ($p > 0.05$).

Galactose was the least in supporting mycelial growth of *P. hepiali* with colony diameter value of 35.6 mm after 14 days from inoculation. However, it was better than cellulose, lactose and control media in terms of colony density. Growth in maltose, mannitol and sucrose were also quite good. The media without carbon source resulted into a thin mycelial mat that was almost translucent (**Figure 4**). Almost the same observation was made on media with cellulose and lactose.

The poor growth in the control media confirms the necessity of a carbon source for the good growth and development of *P. hepiali*. All fungi depend on organic carbon; it is the qualitatively and quantitatively most important nutritional element [22]. Fungi use a wide variety of organic compounds for their carbon requirements. These compounds provide both the structural carbon for all organic compounds as well as providing energy for metabolic processes. The carbon sources include monosaccharides, polysaccharides, organic acids, amino acids, alcohols, and natural products, such as cellulose and lignin [23].

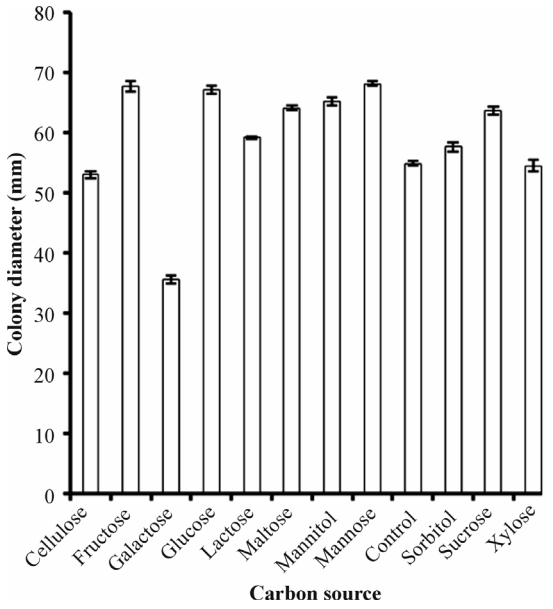

Figure 3. Mycelial growth of *Paecilomyces hepiali* in various carbon sources. Each bar represents the mean value of five replicates measured on the 14th day after inoculation. The error bars represent standard error.

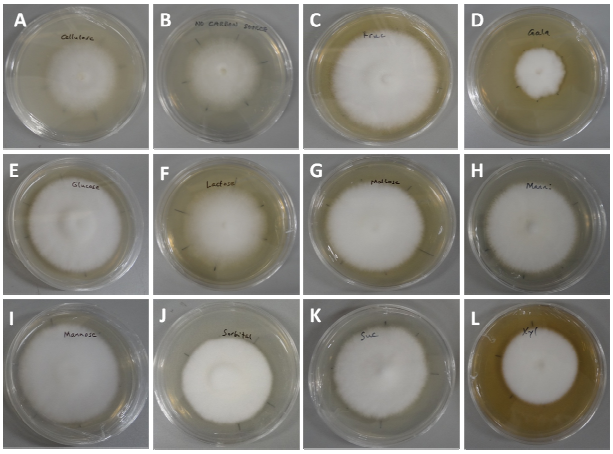

Figure 4. Colonies of *Paecilomyces hepiali* after 14 days of growth in various carbon sources: A-cellulose; B-control (no carbon source); C-fructose; D-galactose; E-glucose; F-lactose; G-maltose; H-mannitol; I-mannose; J-sorbitol; K-sucrose; L-xylose.

In their study with Waterborne Conidial fungi, Sati and Bisht [24] found cellulose to be a poor source of carbon for all the four isolates of the fungi studied. They explained that this could probably be because of lack of extracellular enzyme(s) necessary to degrade cellulose. This could also be the explanation for poor mycelial growth of *P. hepiali* in cellulose. Sung *et al.* [19] reported that the mycelia of *Cordyceps cardinalis* in media with lactose as a carbon source was thinly dense. This is similar to the findings on *P. hepiali* in this study. Lactose is a disaccharide made up of galactose and glucose molecules. In this study, the least colony diameter value was obtained in galactose as a carbon source. Perhaps the poor mycelial growth of *P. hepiali* in lactose was due to the presence of galactose in its structure. However, we do not have an explanation as to why galactose, a simple sugar, was found to be a poor carbon source for mycelial growth of *P. hepiali* in agar media. Imtiaj *et al.* [13] found lactose and galactose to be the most unfavourable carbon sources for various edible mushrooms. Almost similar results are reflected in this study for *P. hepiali.*

3.4. Effect of Nitrogen Source

Among the nitrogen sources tested, peptone, beef extract and yeast extract resulted in the best mycelial growth (**Table 3** and **Figure 5**).

The highest colony diameter (66.06 mm), in 14 days, was obtained in medium with peptone. However, this was not significantly different from values recorded in beef extract (64.60 mm) and yeast extract (64.26 mm). These three organic nitrogen sources produced very well developed aerial mycelia. Medium without nitrogen source produced a very thin and transparent mycelial mat (**Figure 5**). However, the colony diameter was larger than

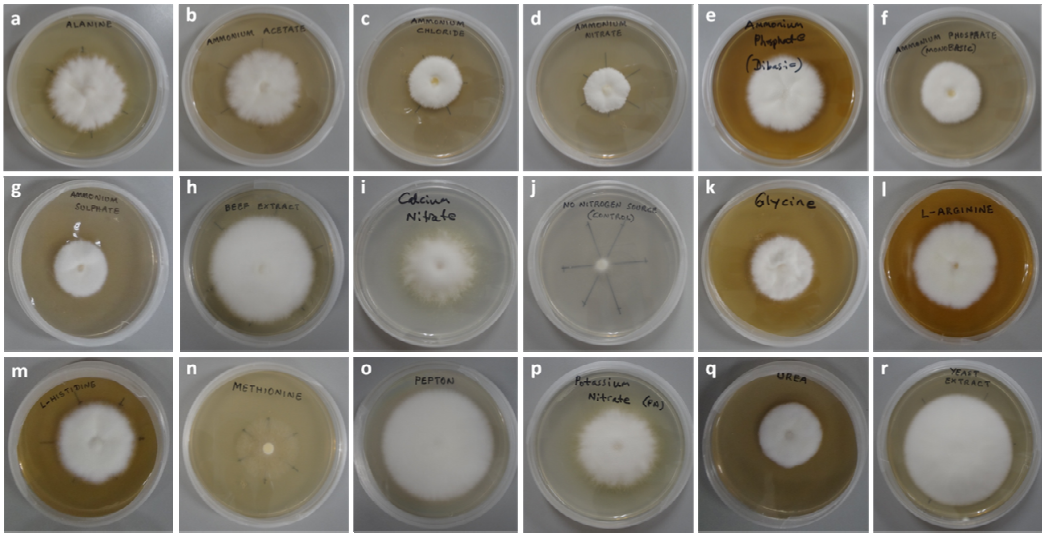

Figure 5. Colonies of *Paecilomyces hepiali* after 14 days of growth in various nitrogen sources: a-alanine; b-ammonium acetate; c-ammonium chloride; d-ammonium nitrate; e-ammonium phosphate, dibasic; f-ammonium phosphate, monobasic; g-ammonium sulfate; h-beef extract; i-calcium nitrate; j-control (no nitrogen source); k-glycine; l-L-arginine; m-L-histidine; n-L-methionine; o-peptone; p-potassium nitrate; q-Urea; r-yeast extract. Each medium was prepared by replacing peptone with 4.0 g of a nitrogen source in the Modified Mushroom Complete Medium (20.0 g glucose, 4.0 g peptone, 1 g K_2HPO_4, 0.5 g $MgSO_4$, 0.5 g KH_2PO_4, 20.0 g agar and 1000 ml distilled water).

Table 3. Effect of nitrogen source on mycelial growth of *Paecilomyces hepialid.*

Nitrogen source	Colony diameter (mm)
Alanine	47.26 ± 1.21^{d}
Ammonium acetate	45.93 ± 1.36^{d}
Ammonium chloride	33.00 ± 1.05^{h}
Ammonium nitrate	27.26 ± 0.64^{i}
Ammonium phosphate, dibasic	46.06 ± 0.43^{d}
Ammonium phosphate, monobasic	37.40 ± 0.86^{ef}
Ammonium sulfate	32.53 ± 1.12^{h}
Beef extract	$\mathbf{64.60 \pm 1.40^{a}}$
Calcium nitrate	46.46 ± 1.17^{d}
Glycine	39.86 ± 0.77^{e}
L-Arginine	50.20 ± 2.09^{c}
L-Histidine	47.86 ± 0.69^{cd}
L-Methionine	33.73 ± 1.62^{gh}
No nitrogen source	54.33 ± 1.06^{b}
Peptone	$\mathbf{66.06 \pm 0.80^{a}}$
Potassium nitrate	54.27 ± 1.74^{b}
Urea	36.00 ± 1.18^{fg}
Yeast extract	$\mathbf{64.26 \pm 0.43^{a}}$

Values in the same column with different letters differ significantly according to Tukey's Multiple Comparisons test ($p < 0.05$). Each value is a mean ± SD of five replicates measured on the 14th day after inoculation. Medium for each nitrogen source was prepared by replacing peptone with 4.0 g of the nitrogen source in the Modified Mushroom Complete Medium (20.0 g glucose, 4.0 g peptone, 1 g K_2HPO_4, 0.5 g $MgSO_4$, 0.5 g KH_2PO_4, 20.0 g agar and 1000 ml distilled water).

all nitrogen sources except for peptone, beef extract and yeast extract (**Table 3**). Methionine resulted in a very thin and almost transparent colony. After fourteen days of incubation, all other inorganic nitrogen sources produced colony diameters ranging from 27.26 mm to 54.27 mm. Among the inorganic nitrogen sources, potassium nitrate produced the best mycelial growth with a well developed aerial mycelium and colony diameter of 54.27 mm.

This experiment has shown that organic nitrogen sources are, by far, more favourable than inorganic nitrogen sources in supporting mycelial growth of *P. hepiali.* This is similar to findings in studies of other fungi. Sung *et al.* [17] reported that nitrogen sources such as yeast extract, peptone and tryptone are favourable for mycelial growth of *O. longissima* as in other Cordyceps and allied species. Dong and Yao [21] pointed out that *C. sinensis* had greater preference on organic nitrogen, which is common in fungi.

3.5. Growth in Rice-Based Media

The results in **Table 4** show that brown rice produced better fruit bodies yield than polished rice. Media supplemented with peptone (*D*1 and *D*2) produced the highest yield of fruit bodies followed by media supplemented with egg yolk (*A*1 and *A*2). Media not supplemented with any egg part or peptone produced the smallest yield of fruit bodies. The findings of this study suggest that addition of an organic nitrogen source to the media enhances fruit body yield of *P. hepiali*. In their study on *Cordyceps*

Table 4. Days to primordia formation and fruit body yield of *Paecilomyces hepiali* in various rice based media.

Media	Days to primordia formation	Fresh weight yield of fruit bodies (g)
*A*1	37	17.4 ± 0.8^{bcd}
*A*2	33	$\mathbf{18.5 \pm 0.7^{ab}}$
*B*1	39	14.2 ± 0.4^{e}
*B*2	35	16.1 ± 0.6^{d}
*C*1	38	16.5 ± 0.4^{cd}
*C*2	34	17.2 ± 0.8^{bcd}
*D*1	33	$\mathbf{18.2 \pm 0.9^{abc}}$
*D*2	31	$\mathbf{19.3 \pm 0.8^{a}}$
*E*1	37	13.2 ± 0.5^{e}
*E*2	36	13.7 ± 0.3^{e}

Values in the same column not sharing same superscript letter are significantly different according to Tukey's Multiple Comparisons test ($p < 0.05$). Each value is a mean ± SD of three determinations.

cardinalis, Kim *et al.* [25] found brown rice to be better than polished rice in size as well as fresh weight of fruit bodies. They also reported that addition of silkmoth pupa and larvae to the media slightly enhanced production of fruit bodies. The higher fruit body yield obtained in brown rice media was not a surprising result considering that brown rice is superior to polished rice in terms of nutrients. As Babu *et al.* [26] indicated, in rice milling, the bran layers and germ removed during polishing are high in fiber, vitamins, minerals as well as protein. Therefore, their removal results in loss of these nutrients.

As shown in **Table 4**, the highest fruit body yield of 19.3 g was obtained in medium *D*2 (40 g brown rice, 0.325 g glucose, 0.65 g sucrose, 2 g peptone and 65 ml corn steep liquor). This was followed by a yield of 18.5 g in medium *A*2 (40 g brown rice, 20 g egg york, 0.32 glucose, 0.65 g Sucrose and 65 ml corn steep liquor). In terms of fresh weight yield, these media were not statistically different ($p > 0.05$). However, if we were to calculate the Biological Efficiency (BE) *D*2 would definitely have a much higher BE than *A*2 because the total mass of ingredients in *A*2 are substantially more than that of *D*2. That makes *D*2 the most suitable medium amongst all the media studied.

Days to primordia formation for all the media ranged from 31 to 39. *D*2 took the least number of days for primordia to start forming and *B*1 took the longest time. The general trend was that the medium with brown rice, in each pair, took fewer days than the one with polished rice in days to primordia formation (**Table 4**).

4. Conclusion

This study found the most favourable temperature range for the growth of *P. hepiali* mycelia to be 25˚C. The mycelial growth response to agar media with various initial pH values did not show remarkable differences. However, pH 6 to pH 9 gave the best growth. Therefore, we recommend this pH for the best growth and development of *P. hepiali* mycelia in agar media. Addition of peptone to cereal grains (lye, wheat, millet, white sorghum, brown rice and oats) agar media is necessary for best mycelial growth of *P. hepiali*. Agars made with powders from grains alone do not support good mycelial growth of this fungus. The general composition of the grain agar, as used in this study, is 20 g grain powder, 4 g peptone, 15 g agar and 1000 ml distilled water. Out of the eleven carbon sources tested, mannose, fructose and glucose were found to be the most favourable for mycelial growth of *P. hepiali*. With regard to nitrogen sources, this fungus preferred organic nitrogen sources namely peptone, beef extract and yeast extract. Brown rice was found to be better than polished rice in yield of fruit bodies of this fungus. The medium with 40 g brown rice, 0.325 g glucose, 0.65 g sucrose, 2 g peptone and 65 ml corn steep liquor was found to be the best in fresh weight yield of *P. hepiali* fruiting bodies. Supplementation of a protein source to the media was found to be a remarkable enhancer of fruit body yield.

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