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Original Article

Nutritional and physical requirements for mycelial growth and basidiocarp production of *Trametes elegans* from the Philippines

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Abstract

This paper highlights the rescue and optimal cultural conditions of the naturally-occurring *Trametes elegans*. This mushroom has white fan-like leathery fruiting body that commonly grows singly on deadwood. The secondary mycelial culture has off-white, velvety texture, and aerial hyphae. Rice bran broth agar with pH ranging from 5.5 to 6.5, incubated in either lighted or dark, at 30°C, and sealed conditions were the optimum nutritional and physical factors for growth. Both cracked corn and sorghum seeds registered as the best substrates for grain spawn production. Four parts of sawdust + six parts of rice straw significantly produced the highest yield (12.03 g) and biological efficiency (2.41%). Enriched cultivation study using supplemented substrates is currently under investigation with the aim to increase the production.

Keywords: *Trametes* spp., Secondary mycelia, Basidiocarp, Philippine wild mushrooms, Optimized culture conditions

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Introduction

Mushrooms are macrofungi with distinct spore-bearing structure called the sporocarp or basidiocarp, which can either be seen hypogeously or epigeously (Chang and Miles, 1992). The existence of many species of naturally-occurring macrofungi in the Philippine is attributable to the presence of massive lignino-cellolusic substrates and the fine climatic conditions. They are commonly found growing on leaf litters, fallen logs and branches, deadwoods, lawns, soil, dead insects, termite's mound, and even on the piles of agro-industrial substrates. However, most of these macrofungi remain relatively unexplored, and their optimal culture conditions necessary to the development of sustainable production techniques are not yet established. It is therefore imperative to continuously collect and domesticate Philippine strains of mushrooms before their extinction because they could be important sources not only of nutritious food, but also of natural medicine. Moreover, mushroom cultivation using practical production technology can generate income for mushroom growers and farmers, which will contribute to strengthening the mushroom industry in the Philippines.

The genus *Trametes* is one of the naturally-occurring macrofungi, and the different species have been reported to inhabit the different areas of the Philippines based on the macrofungal listing. *Trametes corrugata* and *T. versicolor*, for instance, were documented in Angat Watershed Reservation, Bulacan while *T. elegans* was found in Mt. Mingan, Nueva Ecija (Liwanag et al., 2017; Guzman et al., 2018), but their mycelia were not rescued. However, just recently, another wild strain of *Trametes* was also found in Nueva Ecija and was successfully rescued and molecularly identified as *Trametes elegans.* This white rot macrofungus has a thick white, leathery, fan-shaped fruiting body that is naturally growing on deadwoods, fallen logs and branches of trees. Extract of this wild fruiting body contains bioactive compounds and exhibits antibacterial, antioxidant, and cytotoxic effects (Nanglihan et al., 2018).

Most of the reported studies regarding utilization of *Trametes* species have focused on ligninolytic enzyme production such as laccase, synthetic dye decolorization, polysaccharide production, chemical composition elucidation and biological activity evaluation (Trupkin et al., 2003; Tavares et al., 2005a; b; Jing et al., 2007; Cui and Chisti, 2003; Johnsy and Kaviyarasana, 2011; Kamiyama et al., 2013; Aina et al., 2016). Some biological activities of this genus include anti-tumor, antibacterial, anti-oxidant, and anti-proliferative properties exhibited by *T. gibbosa* extract (Ren et al., 2006; Shahbazyan et al., 2017). *Trametes versicolor*, the most famous medicinal species of *Trametes*, has potent anti-human immunodeficiency virus, anti-inflammatory, antioxidant, antigenotoxic and immuno-stimulatory effects (Collins and Ng, 1997; Kamiyama et al., 2013; Knezevic et al., 2015; Chu et al., 2002). This species can also be a source of an antifungal agent against wood-decaying fungi (Teoh and Mashitah, 2012).

Although one work was conducted to optimize the cultural conditions of *T. elegans* for the improved production of antifungal substances in a liquid culture (Liu et al., 2016), optimization of the growth requirements in solid-state conditions is still of our interest since this mushroom grows naturally on solid substrate. Apparently, there are no reports dealing with the optimization of the nutritional and environmental requirements for the growth of mycelial and fruiting body of *T. elegans* in solid-state conditions, which are important parameters in the generation of production technology, hence this work. The aim of the study was to evaluate the growth of mycelia on the different culture media, physical factors, and grain spawning materials, and the production of fruiting body of *T. elegans* using rice straw and sawdust as substrates.

Material and Methods

Source of mushroom and tissue culture

The wild fruiting body of *T. elegans* (Figure 1) growing on the dead trunk of acacia (*Pithecellobium saman* (Jacq.) Benth) was collected from Lingap Kalikasan Park in Central Luzon State University Campus, Science City of Munoz, Nueva Ecija, Philippines. The sample was brought to the laboratory for tissue culture on potato dextrose agar (PDA). Pure cultures were used as inoculants source in the growth performance assays.

Figure-1. Wild fruiting body of *Trametes elegans* on the dead trunk of acacia.

Evaluation of culture media and pH

Four indigenous culture media namely; coconut water agar (CWA), rice bran broth agar (RBBA), corn grit broth agar (CGBA) and potato broth sucrose agar (PBSA) were prepared as described by Dulay et al. (2012a). The 10 mm-diameter mycelial disc from a seven-day old pure culture was inoculated centrally onto each plated medium and incubated at 30°C under alternating light and dark conditions. The mycelial growth rate was determined in five days of incubation. The most favorable medium was adjusted to different pH levels (from 5.0 to 8.0 with 0.5 intervals) using 0.1M NaOH or 0.1M HCL to determine the optimum pH. Each treatment of both set-ups was replicated three times.

Influence of physical factors

Mycelial discs were inoculated on plates containing the best medium at optimum pH and evaluated the three physical factors namely; illumination, temperature and aeration. In illumination, plates were incubated in lighted and total dark conditions. To determine the optimum temperature, plates were incubated at 30ºC, 20ºC, and 10ºC. Finally, in aeration, plates were incubated in sealed and unsealed conditions. The growth rate of mycelia on the different physical factors was recorded. All tests were replicated three times.

Evaluation of spawning materials

In spawn production, three granulated materials such as cracked corn, rice seeds and sorghum seeds were evaluated as substrate for grain spawn production of *T. elegans*. The preparation of grain substrates, inoculation of mycelia and incubation conditions were similar to the procedure of Dulay et al. (2012a). Incubation period and mycelia density were noted. The best grain substrate was determined and used for mass production, which served as inoculant in the evaluation of fruiting body performance.

Evaluation of fruiting body production

Sawdust and rice straw were used as the basal substrate and the different formulations were prepared following the formulations described by Dulay et al. (2017). Fruiting bags were inoculated with 40 g of grain spawn and subsequently incubated at 30°C. Incubation period was recorded. The fully ramified bags were opened at one end and watered three times a day. The yield was recorded, and the percentage biological efficiency was computed.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA). Treatment means were compared using Tukey's HSD at 5% level of significance in The SAS System Version 9.0 (SAS Institute Inc. Cary, NC, USA).

Results

Effect of culture media and pH

The mycelial growth rate on the four culture media in four days of incubation is shown in Figure 2.

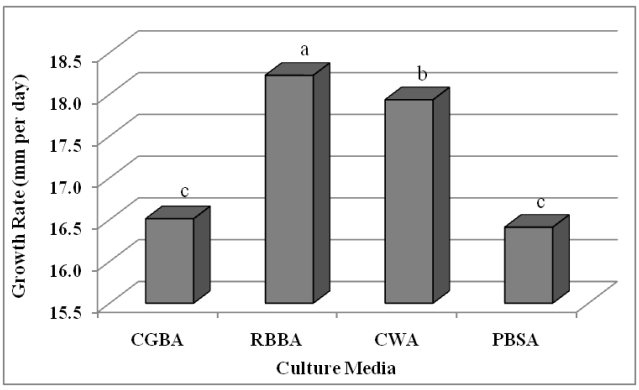

Figure-2. Mycelial growth rate of *T. elegans* on the different culture media; CGBA, corn grit broth agar; RBBA, rice bran broth agar; CWA, coconut water agar; PBSA potato broth sucrose agar at pH 6.0 in four days of incubation. Growth rates with the same letter are significantly comparable from each other at 5% level of significance.

Apparently, mycelia of *T. elegans* responded differently on the four culture media. Among these media, the highest mycelial growth rate was recorded in RBBA, followed by CWA. PBSA registered the lowest growth rate. Very thick mycelia were noted in RBBA, while thin mycelia were observed in CGBA (Figure 3).

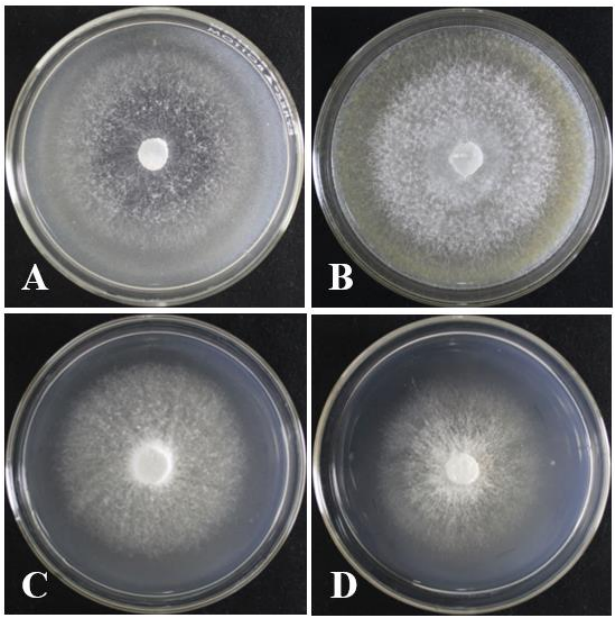

Figure-3. Mycelial growth of *T. elegans* on the different culture media: (A) CGBA, corn grit broth agar; (B) RBBA, rice bran broth agar; (C) CWA, coconut water agar; (D) PBSA, potato broth sucrose agar after four days of incubation.

Notably, the pH levels ranging from 4 to 9 were found suitable for the growth of *T. elegans* mycelia (Figure 4). However, RBBA at pH 5.5 to 6.5 produced the highest growth rate of mycelia after four days of incubation. Mycelia were compact and very thick for all pH levels (Figure 5).

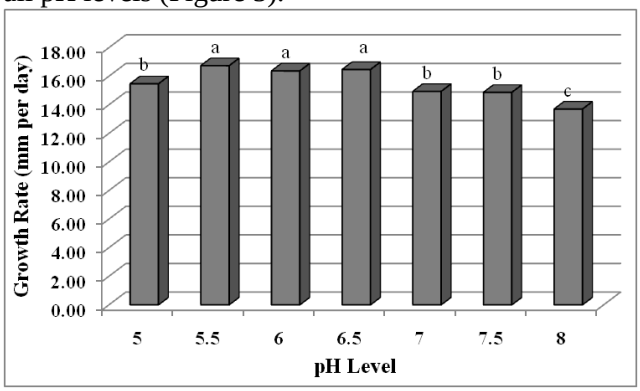

Figure-4. Mycelial growth rate of *T. elegans* on rice bran broth agar at different pH levels in four days of incubation. Growth rates with the same letter are significantly comparable from each other at 5% level of significance.

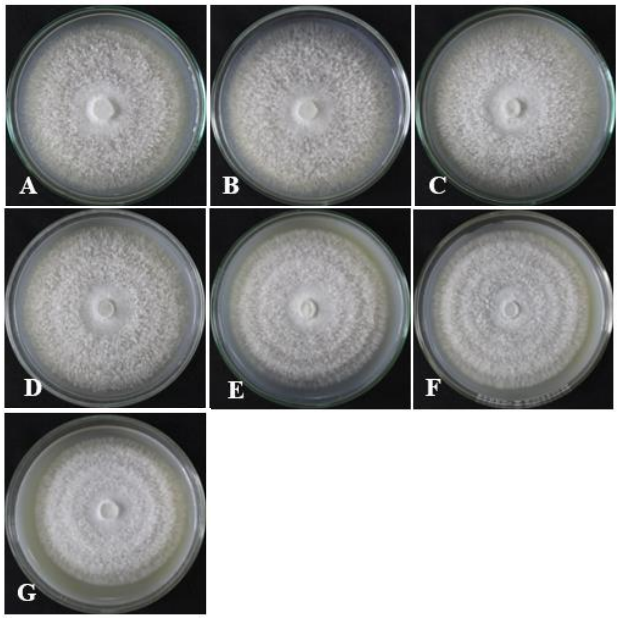

Figure-5. Mycelial growth response of *T. elegans* on rice bran broth agar at different pH levels: (A) 5.0; (B) 5.5; (C) 6.0; (D) 6.5; (E) 7.0; (F) 7.5; and (G) 8.0 after four days of incubation.

Effect of physical factors

The effects of environmental factors such as illumination, temperature and aeration on the growth of *T. elegans* mycelia were studied (Figure 6). In illumination, both artificially lighted and dark conditions were found to be favorable for the mycelial growth of *T. elegans*. However, mycelial density was observed thicker when exposed to lighted condition (Figure 7A). Cultures incubated at 30°C recorded the highest mycelial growth rate and produced very thick mycelia (Figure 7C). The mycelial growth was suppressed when incubated at 20°C, while no mycelial growth was observed in those at 10°C. However, mycelia in sealed plates had higher growth rate and thicker mycelia (Figure 7F) than those in unsealed plates.

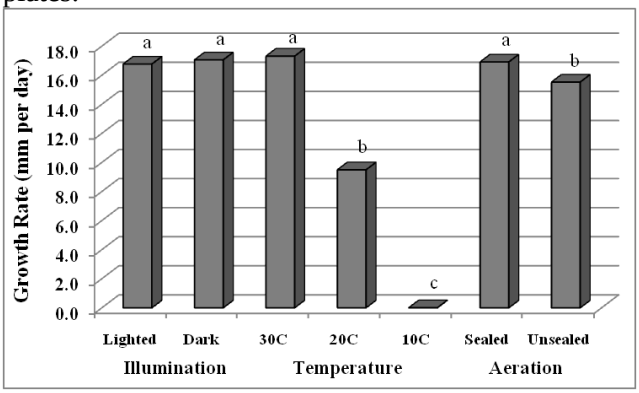

Figure-6. Mycelial growth rate of *T. elegans* on rice bran broth agar with pH 5.5 incubated at different physical factors in four days of incubation. Growth rates with the same letter in each physical factor are significantly comparable from each other at 5% level of significance.

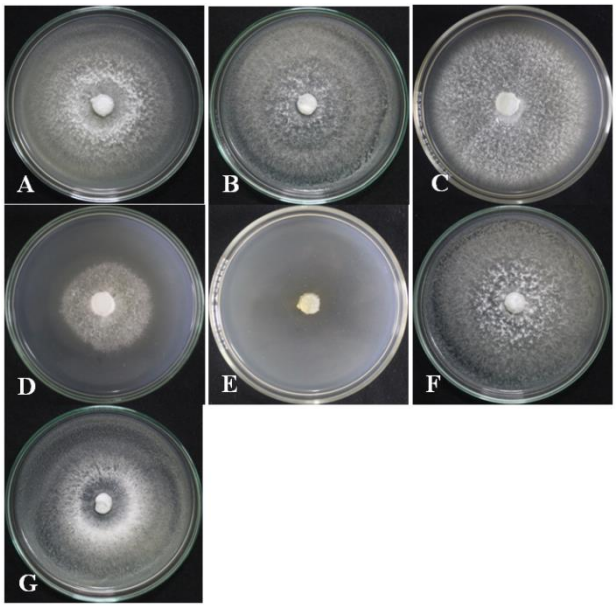

Figure-7. Culture plates of *T. elegans* on rice bran broth agar at pH 5.5 as affected by illumination [(A) lighted and (B) dark], temperature [(C) 30°C, (D) 20°C, and (E) 10°C)] and aeration [(F) sealed and (G) unsealed] conditions.

Grain spawn and fruiting body production

The best granulated materials for grain spawn production was also evaluated (Table 1). Both cracked corn and sorghum seeds significantly recorded the shorter period of incubation (8 days) and showed very thick mycelia. Rice seeds had longer incubation period and thin mycelial density (Figure 8B).

Table-1. Number of days of incubation and mycelia density of *T. elegans* in the three spawn substrate

Grain spawn	Incubation Period (day)	Mycelial density
Cracked corn	8.00 ± 0.00[a]	++++
Rice seeds	9.00 ± 0.00[b]	++
Sorghum seeds	8.00 ± 0.00[a]	++++

Values are mean ± SD of three replicates. Means having the same letter of superscript in the same column are significantly comparable from each other at 5% level of significance. In a column mycelial density: very thin (+), thin (++), thick (+++), very thick (++++).

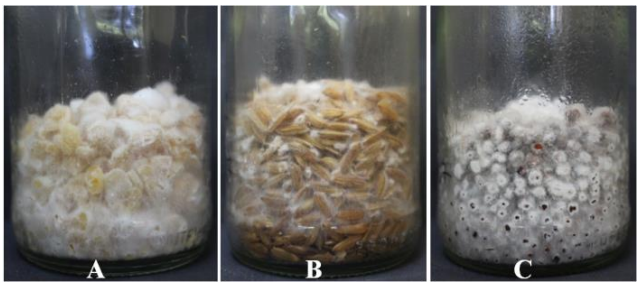

Figure-8. Mycelial ramification of *T. elegans* on different spawning materials: (A) cracked corn, (B) rice seeds, and (C) sorghum seeds 8 days after inoculation.

The most extensive period of incubation, on the other hand, was recorded in pure sawdust. After the full mycelial ramification of the substrate, the emergence and development of fruiting bodies was also observed and documented (Figure 10). From the very thick mycelial density, the primordia appeared 10 days post opening of the substrate bags. These primordia eventually developed into fruiting bodies, expanded and matured. Substrate formulation 4:6 produced the highest yield (12.03 g) with a corresponding biological efficiency of 2.41%, but statistically comparable to 6:4 (10.30 g and 2.06%, respectively).

However, after two days of prolonged incubation, very thick mycelia were also seen in rice seeds spawn. In fruiting body production, the incubation period, yield and biological efficiency in the different substrate formulations were determined (Table 2).

Substrate formulation 8:2 recorded the shortest incubation period, but statistically comparable to 6:4, 4:6, and 2:8. All formulations containing parts of rice straw had very thick growth of mycelia (Figure 9).

Table-2. Number of days of incubation, yield, and biological efficiency of *T. elegans* cultivated in the different formulations of sawdust and rice straw.

Substrate (SD:RS)	Incubation Period (day)	Yield / bag (g)	Biological Efficiency (%)
10:0	30.00 ± 0.00[a]	4.60 ± 0.60[d]	0.92
8:2	26.33 ± 0.58[c]	9.27 ± 0.51[b]	1.85
6:4	26.67 ± 0.58[c]	10.30 ± 1.06[ab]	2.06
4:6	26.67 ± 0.58[c]	12.03 ± 1.93[a]	2.41
2:8	27.00 ± 0.00[c]	7.43 ± 0.90[c]	1.49
0:10	28.00 ± 0.00[b]	6.50 ± 1.10[c]	1.30

Values are mean ± SD of three replicates. Means having the same letter of superscript in the same column are significantly comparable from each other at 5% level of significance.

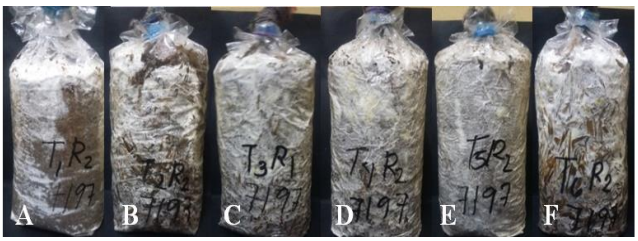

Figure-9. Mycelial growth of *T. elegans* in the different substrate formulations (SD:RS): (A) 10:0, (B) 8:2, (C) 6:4, (D) 4:6, (E) 2:8, (F) 0:10 after 30 days of incubation period.

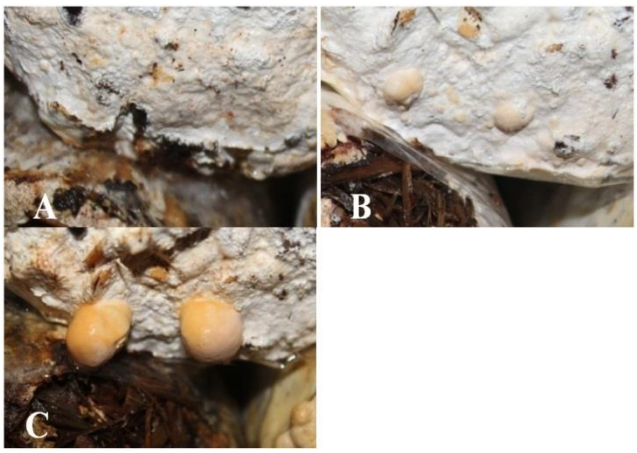

Figure-10. Fruiting body development of *T. elegans*, (A) very thick mycelia coat, (B) formation of primordia, (C) expansion and maturation of fruiting body.

Discussion

The wild fruiting body of *T. elegans* was successfully tissue cultured on PDA plates and was labelled as BIL 7197. However, in order to obtain the most efficient growth, it is necessary to optimize the culture conditions of its mycelia. In the present study, the mycelial growth was evaluated first on the four culture media. RBBA was found to be the most suitable medium that promote efficient mycelial growth of *T. elegans*. This favourable response to RBBA could be accounted to the nutrient compositions of rice bran. Rice bran contains carbohydrates, dietary fiber, sugar, sucrose, glucose, fructose, protein, fats, minerals and vitamins (USDA, 2012).

In screening the suitable media for the six strains of *Coriolus (Trametes) versicolor* from Korea, PDA, yeast extract agar (YEA) and malt extract agar (MEA) produced the most efficient mycelial growth (Jo et al., 2010). However, *T. versicolor* (strain HTV-1) from Mexico has greater preference on MEA than PDA (Guerrero et al., 2011). However, in the previous works, we reported that *Oudemansiella canarii* luxuriantly grew on MEA and PBSA (Dulay and Damaso, 2017), while *Lentinus strigosus* mycelia favored Sabouraud dextrose agar (SDA) and CWA (Dulay et al., 2017). *Polyporus grammocephalus* produced the excellent mycelial growth of on MEA and CGBA (Dulay and Rivera, 2017). Based on the above findings, it is noteworthy to say that each mushroom has unique culture medium preference.

The pH of the medium also influences the growth of mycelia. This study established that the optimum pH for maximum growth of *T. elegans* mycelia was at pH 5.5 to 6.5. Kalaw et al. (2016) reported that most of the studied wild macrofungi from Central Luzon, Philippines had optimal pH at 6 to 7. On the other hand, the favorable pH for mycelial growth of *C. versicolor* and *Ganoderma applanatum* are within pH range of 4 – 6 and 6 – 9, respectively (Jo et al., 2010; Jo et al., 2009). Moreover, the favorable growth of mycelia of *Oudemansiella radicata* was obtained at pH 5 to 9, but pH 6 was the optimum (Kim et al., 2005).

Aside from nutritional requirements, the optimal environmental conditions were also evaluated. Although light is very essential for the growth of mycelia and production of fruiting bodies of mushrooms, it is not the most important factor that influenced the growth of *T. elegans* mycelia, since it was found to luxuriantly grow in either dark or lighted conditions. This corroborates with the study of Kalaw et al. (2016) who reported that out of 13 wild species and strains of Philippine wild macrofungi, mycelial growth of nine mushrooms were favorable in both lighted and dark conditions. Although non-photosynthetic, fungi sense the different qualities and intensities of light (Idnurm and Heitman, 2005). Blue light-emitting diode (LED) was found to be the most effective illumination condition for efficient growth of mycelia and production of basidiocarp of *Lentinus tigrinus* (Damaso et al., 2018). However, one study reported that red and blue LED and dark were favourable for the fast growth of mycelia of *Ganoderma lucidum*.

Temperature is another important physical factor that influences the mycelial growth of mushroom. The superior mycelial growth at 30°C strongly indicates that *T. elegans* is indeed a tropical species. This response is the same to that of *C. versicolor*, which showed optimum temperature at 25-30°C (Jo et al., 2010). Moreover, 32°C was the optimum temperature for the efficient mycelial growth of Philippine mushrooms including *G. lucidum* strains A and B, *L. tigrinus*, *Volvariella volvacea* strain Rang-ayan, *Coprinopsis cinerea* strain Sto. Domingo, and *Lentinus sajor-caju* (Dulay et al., 2012a; Kalaw et al., 2016). These findings imply that the response of mushroom mycelia to temperature is dependent on the species and strain type.

The last physical factor evaluated in the present study was aeration. Apparently, aeration significantly affects the mycelial growth of *T. elegans*. The results of the present work indicate that aeration is one of the most important physical factor that affects the mycelial colonization of *T. elegans*. This favourable response to sealed condition is congruent to the reports of Dulay et al. (2017) and Magday et al. (2014) that sealed condition registered the faster growth of mycelia of *L. strigosus*, and *G. lucidum*. Altogether, this paper demonstrated that the mycelia of *T. elegans* grew efficiently in either lighted or dark, at 30°C, and in sealed condition.

Prior to fruiting body production, it is necessary to determine the best grain spawn material. Grain spawn is a carrier of mycelia that serves as inoculant of fruiting bags for mushroom cultivation. Accordingly, both cracked corn and sorghum seeds were found to be the best substrate for grain spawn production. Rice seeds were also considered a very good substrate. The thin mycelial density in rice seeds at earlier period of incubation could be due to the rice husk that serves as

barrier in penetrating the nutritious part of the grain by the mycelia. The same response in rice seeds was observed in *L. strigosus* and *L. tigrinus* (Dulay et al., 2017; Dulay et al., 2012a).

Fruiting body performance of *T. elegans* was investigated using the different sawdust and rice straw-based formulations. Noticeably, the biological efficiencies of *T. elegans* obtained in this study were very low when compared to the commercially cultivated mushrooms. This case is also the same to that of *T. versicolor* having 3.2% biological efficiency when grown in pure oak sawdust, but increases to 20.3% when cultivated in oak sawdust supplemented with 10% sorghum kernels, 10% wheat bran, and 2% gypsum (Guerrero et al., 2011). Moreover, rice bran improves the biological efficiencies of *Coprinus comatus*, *S. commune*, *G. lucidum*, and *L. strigosus* (Dulay et al., 2012b; Kalaw et al., 2017; Dulay et al., 2017). Accordingly, the effect of supplementation of substrate using rice bran is of great interest to enhance the bio-efficiency of *T. elegans*.

Conclusion

In conclusion, the optimum cultural conditions for luxuriant growth of *T. elegans* mycelia are successfully established in RBBA at pH ranging from 5.5 to 6.5, when incubated in either lighted or dark, at 30°C, and in sealed conditions. Both cracked corn and sorghum seeds serve as the best spawning materials. The fruiting body can be cultivated using formulated sawdust and rice straw as substrate. However, the yield and bio-efficiency are very low, thus, we need to investigate other cultivation techniques such as using enriched substrates and other physical factors in order to improve the biomass yield. Furthermore, the bioactivities and functionalities must also be studied in our intention to establish the role of *T. elegans* in pharmaceutical and nutraceutical field.

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Dulay RMR: Conceptualization of the study, literature review, data interpretation and analysis and manuscript writing
Alcazar AA: Literature review, experimental design, data collection and analysis
Kalaw SP & Reyes RG: Research design and methodology assessment and manuscript critiquing
Cabrera EC: Manuscript critiquing, final reading and approval