IJBPAS, February, 2017, 6(2): 308-315

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

MYCELIAL GROWTH AND FRUITING BODY PERFORMANCE OF THREE PHILIPPINE EDIBLE MUSHROOMS ON VITAMIN A-SUPPLEMENTED MEDIA

RICH MILTON R. DULAY[1*], HANNAH LEI M. HARADA[1], MAORIN MARI R. SANTOS[1], CARLO M. MIGUEL[1] AND MA. ELLENITA G. DE CASTRO[2]

1: Center for Tropical Mushroom Research and Development, Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines

2: Department of Biology, College of Science, De La Salle University, Taft Avenue, Manila, Philippines

***Corresponding Author: E-mail: richmiltondulay@clsu.edu.ph**

Received 10th Sept. 2016; Revised 15th Oct. 2016; Accepted 12th Dec. 2016; Available online 1st Feb. 2017

ABSTRACT

This paper is the first report on the effect of vitamin A supplementation on the mycelial growth and fruiting body production of the three Philippines edible mushrooms. The mycelial growths of *Lentinus tigrinus*, *Schizophyllum commune*, and *Ganoderma lucidum* were stimulated at certain amount of vitamin A in coconut water gulaman (CWG), which peaked at 7.5 mg, 30 mg, and 15.0 mg/100 ml of the media, respectively. The presence of vitamin A in the rice straw-sawdust substrate significantly enhanced the total yield and biological efficiency of *L. tigrinus* (67.76 g and 22.59%) and G. lucidum (12.90 g and 4.30%). However, the fruiting body production of *S. commune* was not affected by vitamin A. Therefore, the effects of vitamin A supplementation on the growth and production of mushroom were dependent on the species and amount or level of vitamin A. Analysis of vitamin A content of the mycelia and fruiting bodies of these mushrooms grown in vitamin A supplemented media is currently under investigation in order to provide information on the possibility of vitamin A fortification in mushrooms, therefore, addressing problems on vitamin A deficiency.

Keyword: Philippine edible mushrooms, vitamin A, mycelia, biological efficiency

INTRODUCTION

Mushrooms are natural sources of nutritionally functional food and physiologically beneficial and non-toxic medicine **[1]**. They contain nutrients such as carbohydrates, proteins, fiber, minerals, vitamins, and essential mycochemicals and exhibited several pharmacological properties including antioxidant, antimicrobial, antiviral, energy boosters and metabolic activators **[2]**. Therefore, there is a great interest in developing fast and efficient technology for mushroom biomass production. Mushroom fruiting bodies are produce using solid substrate like agro-industrial wastes. However, the nutrient compositions of some substrates are inadequate resulting to low biomass yield and biological efficiency. Thus, enrichment of the substrate with supplements like rice bran, molasses, growth hormones and others is necessary in order to improve the yield of mushroom biomass. In the present work, vitamin A was used as supplement of culture media and substrate for mushroom. This vitamin is a type of fat-soluble vitamin, which is essential for normal physiology. In the review conducted by Ames **[3]**, vitamin A has a significant role in nucleic acid metabolism and in increasing resistance to infection.

In this article, we reported the growth promoting activity of vitamin A in the mycelia and fruiting bodies of the three Philippine strains of wood-rooting edible mushrooms namely; *Schizophyllum commune*, *Lentinus tigrinus*, and *Ganoderma lucidum*. These mushrooms are known for their effective pharmacological uses. The effects of vitamin A in the nutrient compositions (especially the amount of vitamin A) and the bioactivities of fruiting bodies and mycelia grown on vitamin A-enriched media are currently under investigation.

MATERIALS AND METHODS

Source of mushroom culture

Pure cultures of *S. commune*, *L. tigrinus* and *G. lucidum* were obtained from the culture collection of the Center for Tropical Mushroom Research and Development, Science City of Munoz, Nueva Ecija, Philippines.

Preparation of culture inoculants

An agar block of approximately 10 mm^2 x 3 mm from each pure culture of the three mushrooms was aseptically inoculated into sterilized potato dextrose agar (PDA) plates and incubated at 30°C to allow mycelia growth. After 7 days of incubation, a flame sterile 10 mm diameter cork borer was used

to prepare mycelial discs as culture inoculant in growth response evaluation.

Preparation of culture media with vitamin A

Coconut water gulaman with varying concentrations of vitamin A were used as culture media in the evaluation of mycelial growth. One liter of coconut water and 20 g of gulaman were combined and boiled until homogenized. Two hundred ml of the medium was prepared for each concentration (7.5 mg, 15 mg, 22.5 mg, and 30 mg). A vitamin A free medium served as the control, 0 mg. Each prepared medium was dispensed in a flask, cotton plugged and properly labeled. These were sterilized in an autoclave at 121°C, 15 psi for 30 min. After sterilization, the different amounts of were mixed to the culture media.

Evaluation of mycelia growth response

The different media were pour-plated and aseptically inoculated with mycelia discs of mushrooms. Triplicate was done per concentration of vitamin A. The inoculated plates were incubated at 30 °C to allow mycelia growth. The daily mycelia growth was measured and the mycelia density was described as very thin (+), thin (2+), thick (3+), very thick or cottony (4+).

Preparation of grain spawn

Two hundred grams of rice seeds were boiled until swelling and slit opening of the husk attained. After which, seeds were air-dried until 65% moisture content was reached, and 40 g of boiled seeds were dispensed into polypropylene plastics plugged with cotton and wrapped with recycled paper. These were sterilized in an autoclave at 15 psi, 121°C for 30 min and aseptically inoculated with mycelia discs of mushroom. Grain spawn were incubated at 30°C for 10 days which served as the inoculant of the fruiting bags containing substrate infused with vitamin A.

Evaluation of fruiting body production

The effect of the presence of vitamin A in the substrate to the fruiting body production of *S. commune*, *L. tigrinus* and *G. lucidum* was also investigated. Five hundred grams of formulated substrate (7 parts rice straw and 3 parts sawdust by volume) was compacted in a polypropylene plastic. Each bag was infused with 60 mg of vitamin A in 300 g of substrate with 5 replicates for each mushroom species. Bags without vitamin A served as the control. These were individually provided with opening using cut pvc pipe, plugged with cotton, covered with recycled paper, and sterilized at 15 psi, 121 °C for 45 minutes. After cooling, each bag

was inoculated with grain spawn and subsequently incubated at 30 °C in the incubation room. Once completely colonized with mycelia, fruiting bags were transferred into the growing house with 80-90% RH to allow fruiting body development. The fruiting bodies were harvested, weighed and the biological efficiency was computed. Data were analyzed using analysis of variance (ANOVA) and DMRT was used to determine the significant treatment comparison at 5% level of significance.

RESULTS AND DISCUSSION

Mycelial growth response of the three mushrooms

In mushroom production, pure culture of mushroom mycelia serves as the source of cell lines for production of biomass. Thus, it is necessary to determine the nutritional requirements for growth. In this study, the mycelial growth responses of *S. commune*, *L. tigrinus* and *G. lucidum* on the coconut water gulaman (CWG) with varying amounts of vitamin A were evaluated. Table 1 presents the mycelial growth diameter and mycelia density of the three mushrooms on the evaluated media after 4 days of incubation. The plate cultures of the three mushrooms are shown in Figure 1. Apparently, the three mushrooms response differently to the varying amounts of vitamin A. In *L. tigrinus*, the widest mycelial growth diameter was observed in CWG with 7.5 mg of vitamin A with 80.90 mm, which was statistically comparable with CWG having 15.0 mg of vitamin A (80.63 mm). The lowest mycelial diameter was noted in CWG with 30.0 mg having 76.27 mm. On the other hand, the mycelial growth of *S. commune* had increased in increasing amount of vitamin A. The vitamin A-free CWG recorded the lowest diameter whereas CWG with 30 mg of vitamin A registered the widest mycelial diameter. However, in *G. lucidum*, the widest mycelial diameter was peaked in CWG with 22.5 mg of vitamin A while the lowest diameter was observed in CWG with 30 mg of vitamin A. These results strongly indicate that the effect of vitamin A on the mycelial growth was species dependent and amount of vitamin A dependent. Mycelial growth was stimulated at certain amount of vitamin A in specific species of mushrooms. The presence of vitamin A in CWG did not affect the mycelial density of the three mushrooms. Vitamin A is a fat soluble vitamin that is a powerful antioxidant **[4]**. The retinol palmitate has important biological roles in the fungal metabolism and some are considered beneficial at certain concentration **[5]**. Vitamin A is needed to form gylocoprotein, a combination of sugar and

protein, which help the cell growth **[4]**, this is almost certainly one of the cause of the rapid growth of the mycelia.

Fruiting body production of the three mushrooms

The present study also evaluated the effect of vitamin A on the fruiting body production and biological efficiency of the three mushrooms. To carry out this, vitamin A (60 mg) was infused in 300 g of the substrate in a fruiting bag and inoculated with grain spawn of the three mushrooms. The results of total yield and the computed percentage biological efficiency were presented in Table 2. Aside from the mycelia response, it can also be noted that vitamin A affects the fruiting body production of the three mushrooms evaluated. In *L. tigrinus*, vitamin A supplementation in the substrate significantly enhanced the fruiting body production with higher total yield of 67.76 g (22.59% biological efficiency). However, vitamin A supplementation did not significantly influence the fruiting body production of *S. commune*. *G. lucidum* grown in vitamin A-supplemented substrate recorded the higher total yield (12.90 g) and biological efficiency (4.30%). No fruiting body was noted in *G. lucidum* grown in vitamin A-free substrate.

The fruiting bodies of the three mushrooms grown on substrates with and without vitamin A supplementation are shown in Figure 2. The results the study clearly dictates the important role of vitamin A on the physiology of fruiting body development of mushrooms, which we need to investigate in the next study.

Table 1. Mycelial growth diameter and density of *G. lucidum*, *L. tigrinus* and *S. commune* on coconut water gulaman at varying concentrations of vitamin A after 4 days of incubation

Mushroom	Amount of vitamin A (mg / 100 ml)	Mycelial Growth Diameter (mm)	Mycelial density
L. tigrinus	0.0	79.53^{b}	3+
	7.5	80.90^{a}	3+
	15.0	80.63^{a}	3+
	22.5	78.23^{b}	3+
	30.0	76.27^{c}	3+
S. commune	0.0	75.09^{d}	3+
	7.5	76.11^{d}	3+
	15.0	78.52^{c}	3+
	22.5	80.69^{b}	3+
	30.0	82.90^{a}	3+
G. lucidum	0.0	81.85^{c}	3+
	7.5	83.11^{b}	3+
	15.0	85.82^{a}	3+
	22.5	85.63^{a}	3+
	30.0	79.77^{d}	3+

Treatment means in each mushroom species with the same superscript are not significantly different from each other at 5% level of significance using DMRT. In mycelial density column, very thin (+), thin (2+), thick (3+), very thick or cottony (4+)

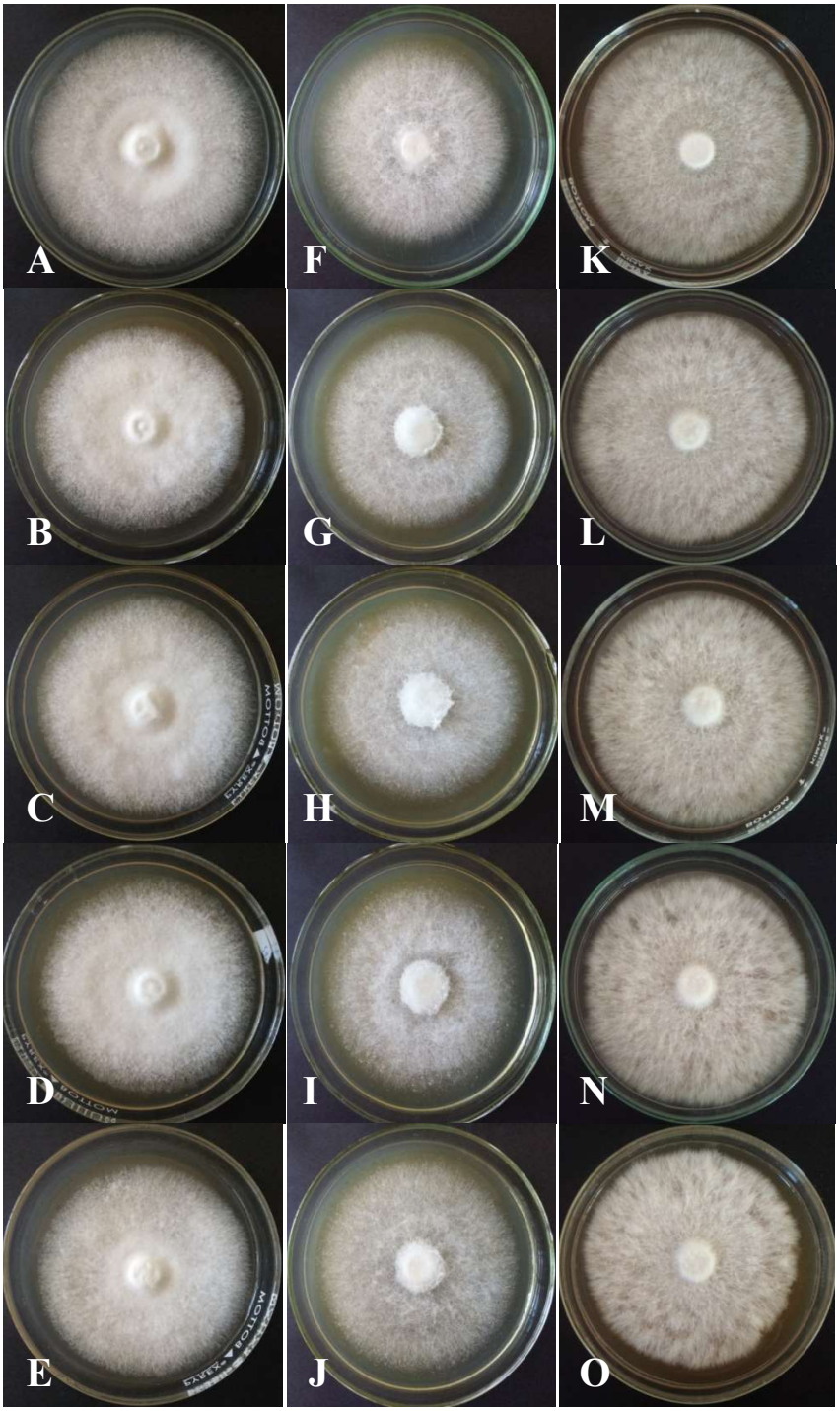

Figure 1: Plate cultures of *L. tigrinus, S. commune, G. lucidum* (left to right) on coconut water gulaman supplemented with varying amounts of vitamin A: 0 mg (A, F, K), 7.5 mg (B, G, L), 15 mg (C, H, M), 22.5 mg (D, I, N), and 30mg (E, J, O)

Table 2: Total yield and biological efficiency of the three mushrooms grown on rice straw-sawdust formulation supplemented with vitamin A			
Mushroom	**Substrate**	**Total yield (g)**	**Biological efficiency (%)**
L. tigrinus	**Vit. A free**	**18.68**	**6.22**
	Vit. A supplemented	**67.76***	**22.59***
S. commune	**Vit. A free**	**13.24**	**4.41**
	Vit. A supplemented	**9.54**	**3.18**
G. lucidum	**Vit. A free**	**0.00**	**0.00**
	Vit. A supplemented	**12.90***	**4.30***
Asterisks (*) indicate significant difference of means at 5% level of significance using t-test.			

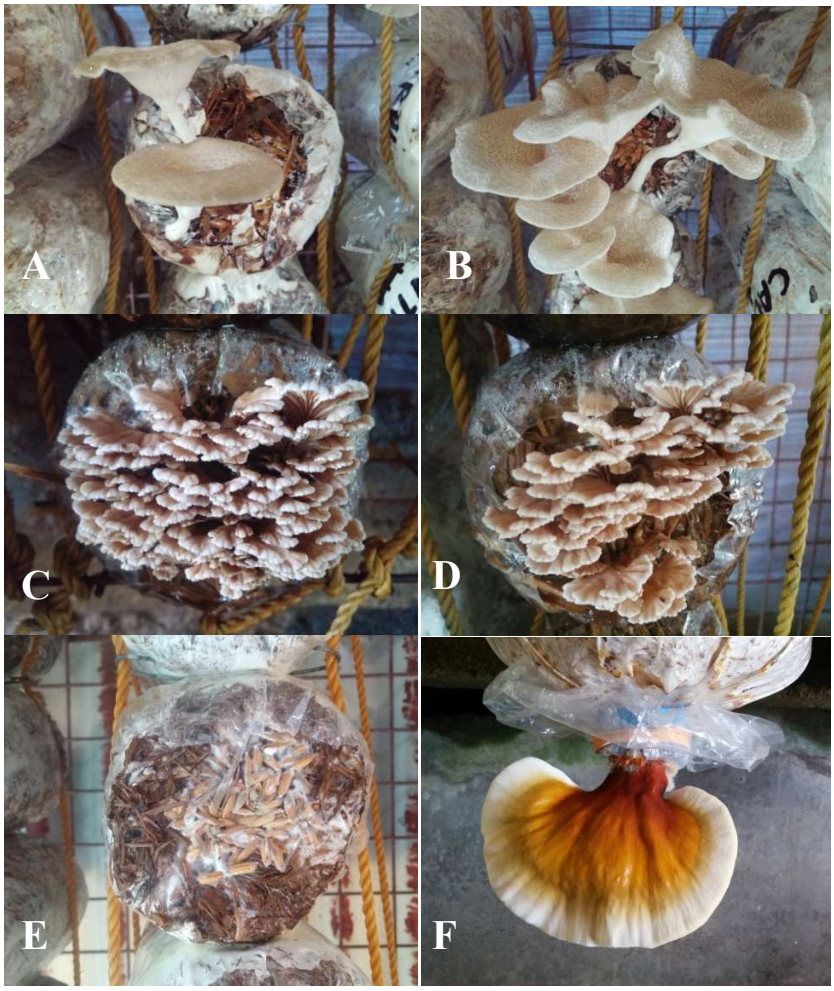

Figure 2: Effect of vitamin A supplementation [vitamin A free (left) and vitamin A supplemented (right)] on the fruiting body production of *L. tigrinus* (A, B), *S. commune* (C, D) and *G. lucidum* (E, F).

CONCLUSION

To the best of our knowledge, no work was done in investigating the effects of vitamin A on the fruiting body production of any mushrooms. This study, therefore, is the first report. This work demonstrated the effect of vitamin A supplemented in the culture media and substrate on the mycelial growth and fruiting body production of the three Philippines edible mushrooms namely; *L. tigrinus*, *S. commune*, and *G. lucidum*. The effects of this supplement were dependent on

the mushroom species and amount or level of supplementation. Composition analysis on the level of vitamin A in the mycelia and fruiting bodies of Philippine mushrooms grown in vitamin A supplemented media is currently under investigation in order to provide information on the possibility of vitamin A fortification in mushrooms, therefore, addressing problems on vitamin A deficiency.

REFERENCES

[1] Wasser SP, Weis AL. Therapeutic effects of substances occurring in higher basidiomycetes mushrooms: a modern perspective. Critical Review of Immunology, 1999, 19(1), 65-96.

[2] De Silva DD, Rapior S, Hyde KD, Bahkali AH. Medicinal mushrooms in prevention and control of diabetes mellitus. Fungal Diversity, 2012, 56, 1-29.

[3] Ames SR. Fat-soluble vitamins. Annu. Rev. Biochem, 1958, 27, 371-402.

[4] Embree ND. Fat-Soluble Vitamins. Annu. Rev. Biochem, 1947, 16, 323-358.

[5] Weete J. Fungal Lipid Biochemistry: Distribution and Metabolism. Department of botany and Microbiology. Auburn University. Auburn, Alabama. Plenum Press. New York and London. 1974.