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Influence of culture conditions on mycelial growth and luminescence of *Panellus stipticus* (bull.) P. Karst.

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ABSTRACT:

Panellus stipticus (Bull.) P. Karst, a naturally bioluminescent tropical fungus, has been studied in vitro for the optimum culture conditions viz culture media, temperature, pH and days of incubation required for luminescence. Temperature and pH affect growth and bioluminescence to a great extent. Glucose-peptone medium has been found to be the best for optimum mycelial growth as well as luminescence. The fungus exhibits luminescence at 20-24°C. The maximum mycelia dry weight (mg/25ml of the basal media) and luminescence observed at pH 4.0. The fungus exhibits luminescence after eight days of incubation at 24°C and pH 4.0, whereas it intensified to maximum after 13 days of incubation (pH 4.0 and temperature 24°C).

Keywords:
Bioluminescence, *Panellus stipticus*, culture conditions, mycelial growth.

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INTRODUCTION

Distributed throughout the world, the numerical strength of bioluminescent fungi have been estimated to be 42 species within nine genera by Wassink (1978) and 64 species belonging to three evolutionary lineages by Desjardin *et al.,* (2008). More than a half of these species are native to Australia and Asia, belonging to the genus Mycena. The bioluminescent basidiomycetes can be used to evaluate the acute toxicity of either pure toxicants or environmental samples (Weitz *et al.,* 2002). For the above purpose and to improve the sensitivity of the method, culture conditions must be investigated to ensure maximal emission.

An attempt has been made to find out the effect of culture conditions for the optimum mycelia growth and bioluminescence of *Panellus stipticus* (Bull.) P. Karst.

MATERIALS AND METHODS

Culture and Culture conditions: The cultures of *Panellus stipticus* (Bulliard ex Fr.) Karst have been obtained from the Lux Biotech. Ltd. U.K. The pure cultures of *Panellus stipticus* have been maintained on malt yeast agar medium. (malt extract 3g, yeast extract 3g, peptone 5g, glucose 10g, agar 20g in distilled water to make 1 litre). For all experiments, the fungi are sub-cultured onto the above mentioned media and stored for up to one month at ±4°C in the refrigerator.

Procedure:

Each flask, containing 25 ml of the basal medium was inoculated with one ml of the standardized mycelial suspension, shaken well and allowed to germinate for five hours at optimum temperature of each fungus to remove lag effect and then incubated at the respective optimum temperature of each fungus for optimum days in still culture. For solid media experiments, a mycelial disc was seeded in 50 ml of the solid medium in 250 ml Erlenmeyer conical flask. Three replicates were kept for each variable.

Preparation of culture media and inoculation:

Glucose-peptone medium (Glucose 10.0 g/l, Peptone 2.0 g/l, potassium dihydrogen orthophosphate 1.0 g/l, magnesium sulphate 0.5 g/l and distilled water to make 1000 ml) was used in all the experiments for *Panellus stipticus* as the medium gave the optimum luminescence of the fungus. The inoculum for experiments on growth and luminescence in relation to temperature, pH and days of incubation was prepared by grinding mycelium (5 days old) growing in optimal basal medium under optimum conditions of each fungus aseptically to get concentrated mycelia suspension which was diluted to get 2.5 mg of dry weight of mycelium/ml of the suspension.

Basal media:

The fungus was grown in twelve different liquid basal media (viz. Raulin's, Richard's, Dox's, Coon's, Czapek's-I, Czapek's-II, Brown,s-I, Brown,s-II, Glucose -nitrate, Glucose-peptone, Asthana & Hawker's and Elliot's medium) to know the best medium for their growth and luminescence. The initial pH of all the different media was not changed and checked before and after autoclaving at 15 lbs psi steam pressure for 15 minutes. Twenty five ml of the basal media were apportioned in each 100 ml sterilized Erlenmeyer conical flask aseptically and seeded with one ml of the standardized mycelial suspension and incubated for 15 days (taken tentatively). Three replicates were kept for each basal medium and the fungus. These fungi were incubated at 22°C.

Temperature:

The effect of temperature on mycelia growth and bioluminescence was studied by growing the fungus on basal medium (glucose-peptone) at 16°C, 20°C, 24°C, 28°C and 32°C. The temperature of the incubator was checked and recorded every day.

H-ion concentrations:

A hydrogen-ion concentration range of 3.0-9.0 was selected to observe the growth and luminescence of

the fungus with a difference of unit pH. The basal medium was apportioned in to 7 aliquots and sterilized at 15 lbs psi steam pressure for 15 minutes in separate flasks. The pH of each aliquot was adjusted to a separate unit value aseptically with sterilized solution of N-HCl and N-KOH and checked over P/L Philips precision instruments PR 9045 M. Twenty five ml of the basal media were apportioned in each 100 ml sterilized Erlenmeyer conical flask aseptically and seeded with one ml of the standardized mycelial suspension and incubated at corresponding optimum temperatures 24°C for 15 days (taken tentatively). Three replicate were kept for each H-ion concentrations.

Days of Incubation:

A period of 30 days was selected to observe the optimum days of incubation for the growth and luminescence of the fungus. The basal medium (Glucose -peptone) was adjusted to an optimum pH of 4.0 with sterilized solution of 1N KOH after sterilization at 15 lbs psi steam pressure for 15 minutes. Twenty five ml of the basal media were apportioned in each 100 ml sterilized Erlenmeyer conical flask aseptically and seeded with one ml of the standardized mycelial suspension and incubated for 30 days at their corresponding optimum temperatures 24°C. Three replicate were kept for each variable.

Determination of mycelial dry weight and final pH: At the end of each experiment, the mycelium of each replicate was filtered through a goosch crucible of known weight (containing a disc of Whatman filter paper No.1). The mycelium was dried at 60°C in a hot air oven to a constant weight and the weight of the individual replicate was recorded. The final pH of the culture filtrate of the individual replicate was checked over Philips precision instrument PR 9045 M and recorded.

Statistical analysis of the data:

The data on growth of all the experiments were analysed statistically with respect to dry weight of the mycelium of individual replicate with variables by applying one-way Anova in terms of significance and non-significance of the data. The significance is denoted by statistical error (S.E.) and Statistical error of difference (S.Ed.)

Bioluminescence measurement:

Bioluminescence has been measured by using a TD 20/20 (Version 2.2) luminometer with the sensitivity set at 35% and recorded as Relative Light Units (RLU). All bioluminescence measurements have been made at the same time.

RESULTS AND DISCUSSION

Liquid/solid basal media experiment:

In the present study, Glucose-peptone medium has been found to be an optimum medium for the luminescence of *Panellus stipticus* **(Plate-1)**. The final H -ion concentration of the basal media did not change significantly with the growth of the fungus. The fungus was observed to form incomplete, submerged, dull white mycelial mass with Raulin's and Richard's medium; incomplete, submerged, cottony white mycelial mass with Dox's, Coon's, Czapek's-I, Czapek's-II, Glucose-nitrate and Asthana & Hawker's medium; Brown's-I and Brown's-II medium showed dirty white mycelial mass. It forms incomplete, superficial as well as submerged, yellowish white mycelial mass with Glucose-peptone medium. The fungus shows no growth in Elliot's medium. The data on average mycelial dry weight plotted against different basal media are depicted in **Fig. 1.** The data on growth of each fungus were analysed statistically in terms of standard error (S.E.) and standard error of difference (S.Ed.)

Temperature relationship:

A temperature of 24°C is optimum for the growth and luminescence **(Plate-2).** The final pH of the basal medium does not change to a significant level by the mycelia growth. The fungus forms incomplete, superficial as well as submerged, cottony white mycelial mass at 16°C, 20°C, 24°C, and 28°C whereas it forms

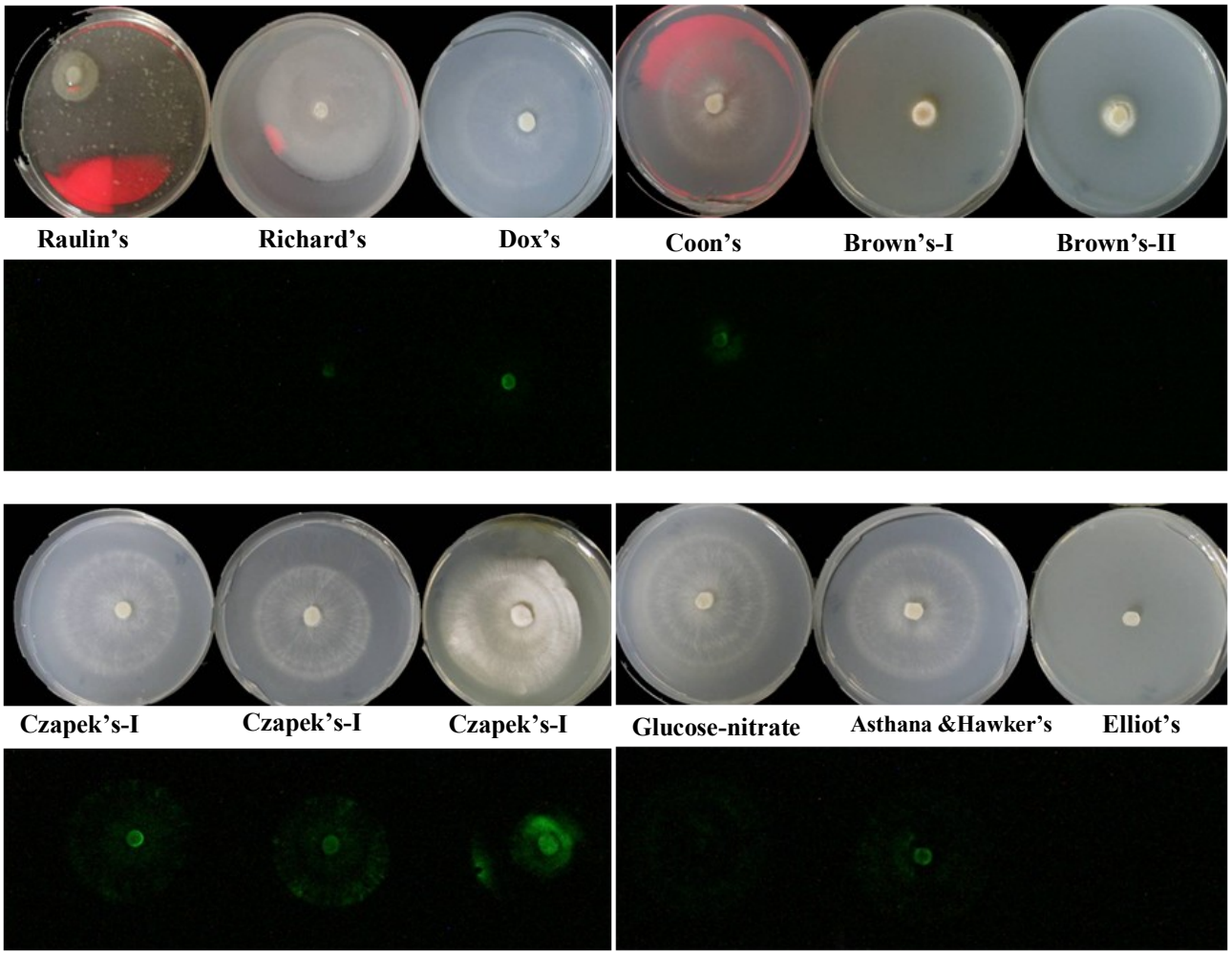
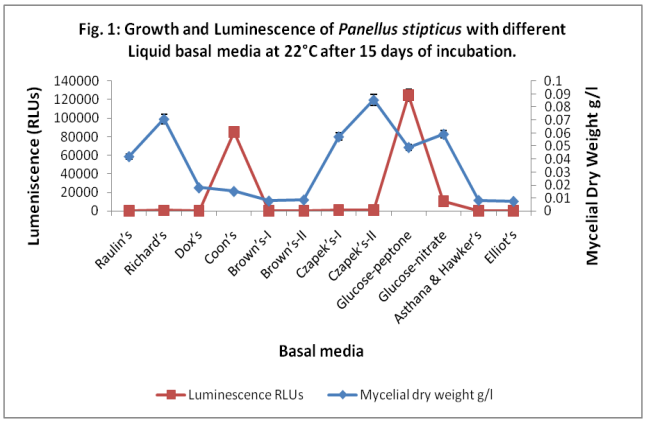
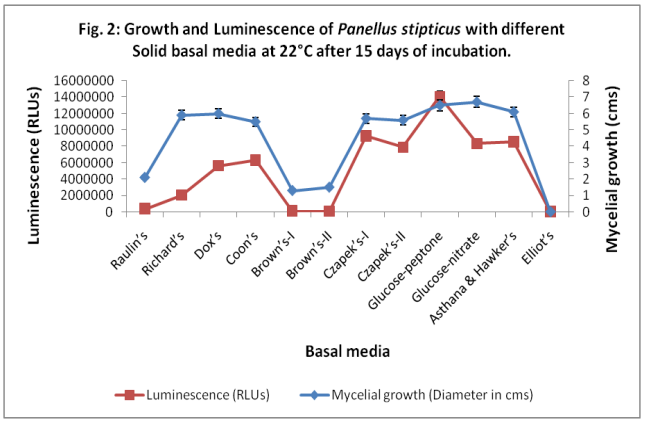


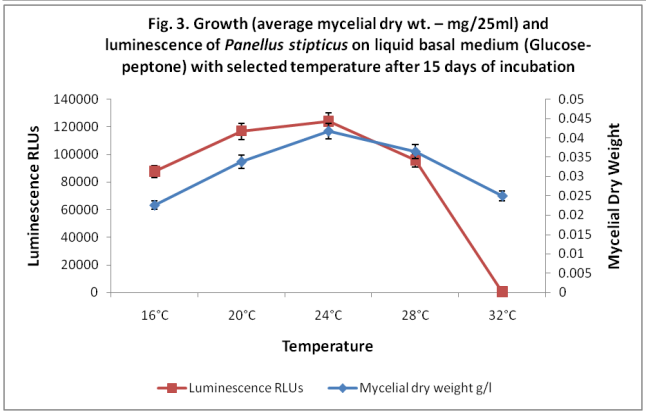
Plate No. 1 Luminescence (RLUs) in natural light exposure and dark condition of *Panellus stipticus* with different solid basal media at 22°C after 15 days of incubation.

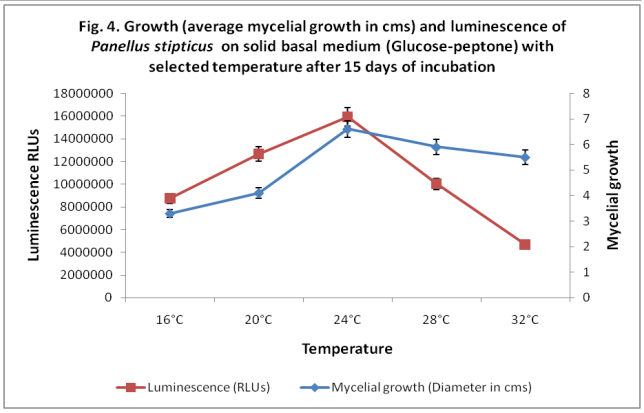




small, submerged, dirty white mycelial mass at 32°C. The data on average mycelial dry weight/mycelial diameter and luminescence plotted against different temperature are depicted in **Figs. 3 & 4.**

It has been noted that temperature affects mycelia growth and bioluminescence of naturally bioluminescent fungi (Bermudes *et al.,* 1990; Berliner, 1961; Buller, 1924; Ya-li Lv, *et al.,* 2010 and Airth *et al.,* 1966). Buller 1924 found an optimum temperature between 10°C and 25°C and a maximum temperature of 35-37°C for the luminescence of *P. stipticus*. Bermudes *et al.,* 1990 and Berliner, 1961 reported the optimum





temperature for luminescence of *P. stipticus* at 22°C and 18-26°C, respectively. Weitz *et al.,* 2001, 2002, reported 22°C as the optimum temperature for both mycelia growth and luminescence of *P. stipticus*. The optimum temperature for the growth of basidiomycetes has been reported between 20°C and 30°C (Boddy, L, 1983). The temperature optima for mycelia growth and bioluminescence that were found in this study for *P. stipticus* were similar to those in previous studies, but they have not previously been done on liquid medium.

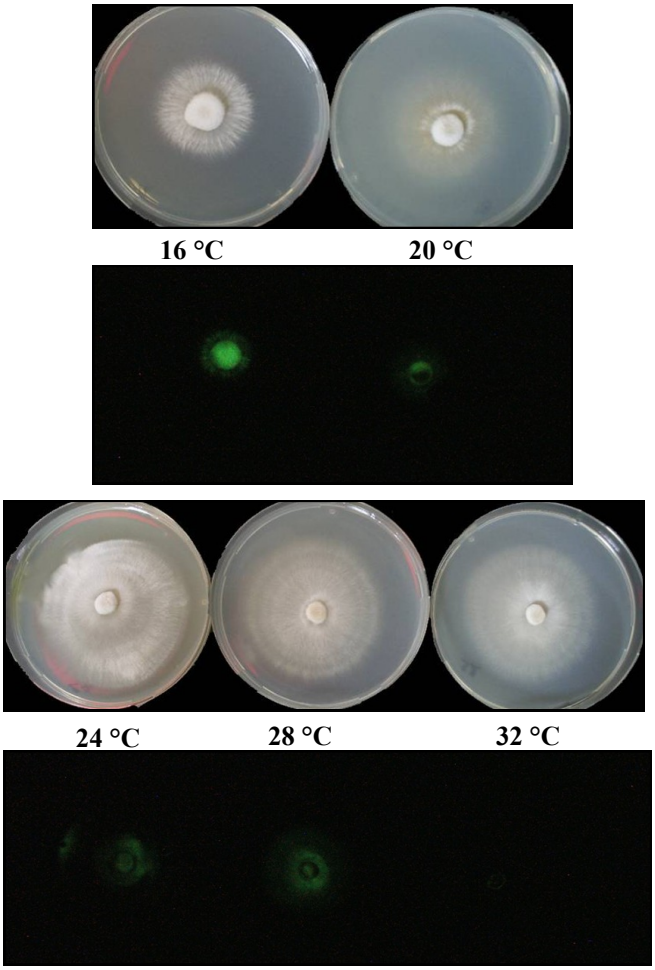


Plate No. 2 Bioluminescence (RLUs) of *Panellus stipticus* with selected temperature after 15 days of incubation (Solid).

Hydrogen-ion concentration relationships:

The study reveals that the optimum H-ion concentrations for the growth and luminescence is recorded at 4.0. The mean final pH of the culture filtrate does not change to a significant level with the growth of the fungus. The fungus forms incomplete, submerged as well as superficial, cottony white mycelial mass at pH 3.0-6.0 whereas it shows no growth at pH 7.0-9.0. The data on average growth and luminescence plotted against H-ion concentrations are depicted in **Figs. 5 & 6.**

Bermudes *et al.,* 1990 reported that *P. stipticus* had a bioluminescence optimum of pH 3.8, with poor growth and luminescence below pH 3.6 or above pH 4.4 and no growth or luminescence at pH 6.0 or above. Weitz *et al.,* 2001 reported optimum pH 3.5-4.0 for the growth of *P. stipticus* and poor mycelial growth at pH 4.5 and above. The optimum pH for mycelial growth and luminescence were found to be similar to those in previous study but these studies have been reported only on solid medium and not on defined medium.

Days of incubation relationship:

Maximum luminescence is exhibited by the fungus after 13 days of incubation in liquid medium whereas in solid medium after 13 days of incubation it decreases and then increases after 18 days and optimum luminescence is attained after 24 days of incubation.

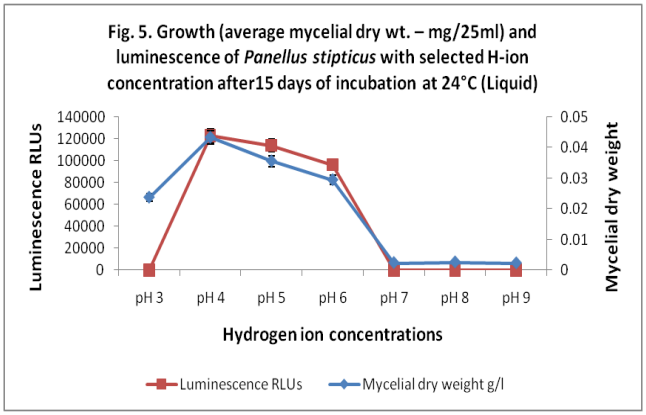


Fig. 5. Growth (average mycelial dry wt. – mg/25ml) and luminescence of *Panellus stipticus* with selected H-ion concentration after15 days of incubation at 24°C (Liquid)

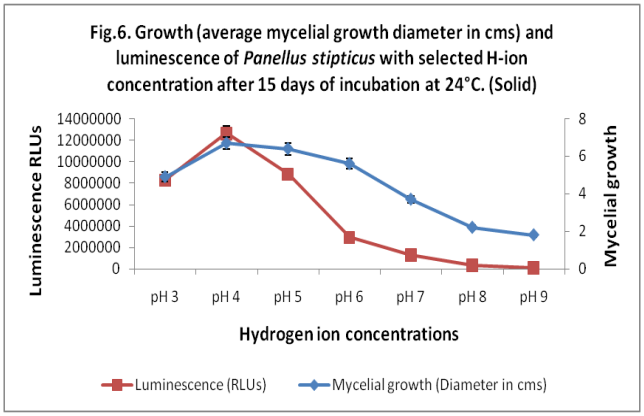


Fig.6. Growth (average mycelial growth diameter in cms) and luminescence of *Panellus stipticus* with selected H-ion concentration after 15 days of incubation at 24°C. (Solid)

The growth was declined after optimum days of incubation in the fungus. The final pH of the medium does not change to a significant level up to the optimum days of incubation, thereafter it starts increasing in its growth.

The fungus forms incomplete, submerged, white mycelial mass up to the 7th day of incubation. It forms superficial as well as submerged, cottony white mycelial mass from 8th – 18th days of incubation and complete, thick, superficial as well as submerged, yellowish white mycelial mass from 19th – 30th day of incubation. The data on average mycelial growth and bioluminescence plotted against days of incubation are depicted in **Figs. 7&8.**

Weitz *et al.,* 2001, reported that the luminescence occur after 8 days in *P. stipticus* on solid medium.

CONCLUSION:

The naturally bioluminescent tropical fungus *Panellus stipticus* has been studied for the optimization of culture conditions (culture media, temperature, pH and days of incubation). Pure culture of *Panellus stipticus* was obtained from Lux Biotech. Ltd. U.K. and stock culture has been maintained on malt yeast agar medium at 0-4°C and revived after two months. Glucose-peptone medium containing g/l: Glucose 10.0 Peptone 2.0, potassium dihydrogen orthophosphate 1.0, magnesium sulphate 0.5, has been used for the studies on the luminescence of *Panellus stipticus* in all the experiments. The optimum temperature for the growth and luminescence of the fungus is 24°C. The optimum H-ion concentration for the growth and luminescence of the fungus is 4.0. The fungus attains maximum luminescence after 13 days of incubation in liquid medium whereas in solid medium after 13 days of incubation it decreases and then increases after 18 days of incubation and attains optimum luminescence after 24 days of incubation.

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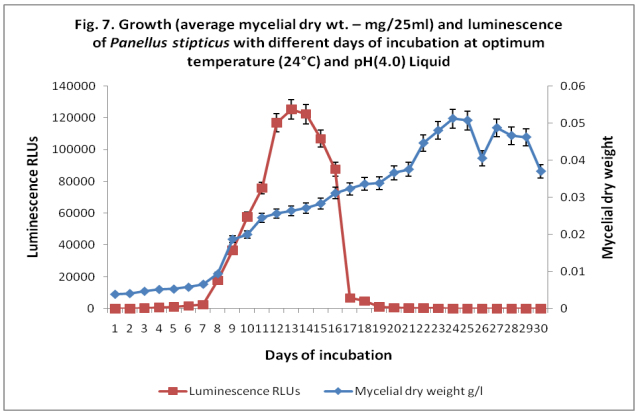

Fig. 7. Growth (average mycelial dry wt. – mg/25ml) and luminescence of *Panellus stipticus* with different days of incubation at optimum temperature (24°C) and pH(4.0) Liquid

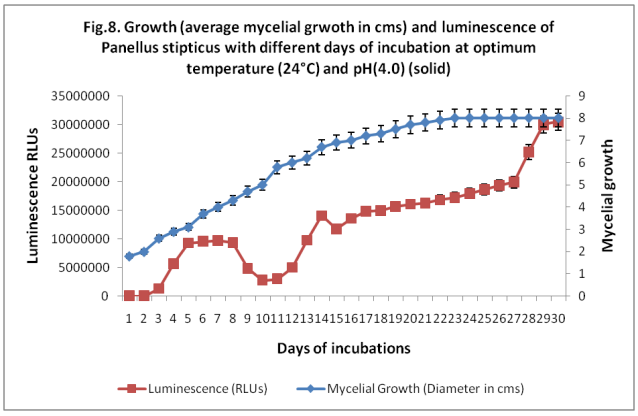

Fig.8. Growth (average mycelial grwoth in cms) and luminescence of Panellus stipticus with different days of incubation at optimum temperature (24°C) and pH(4.0) (solid)

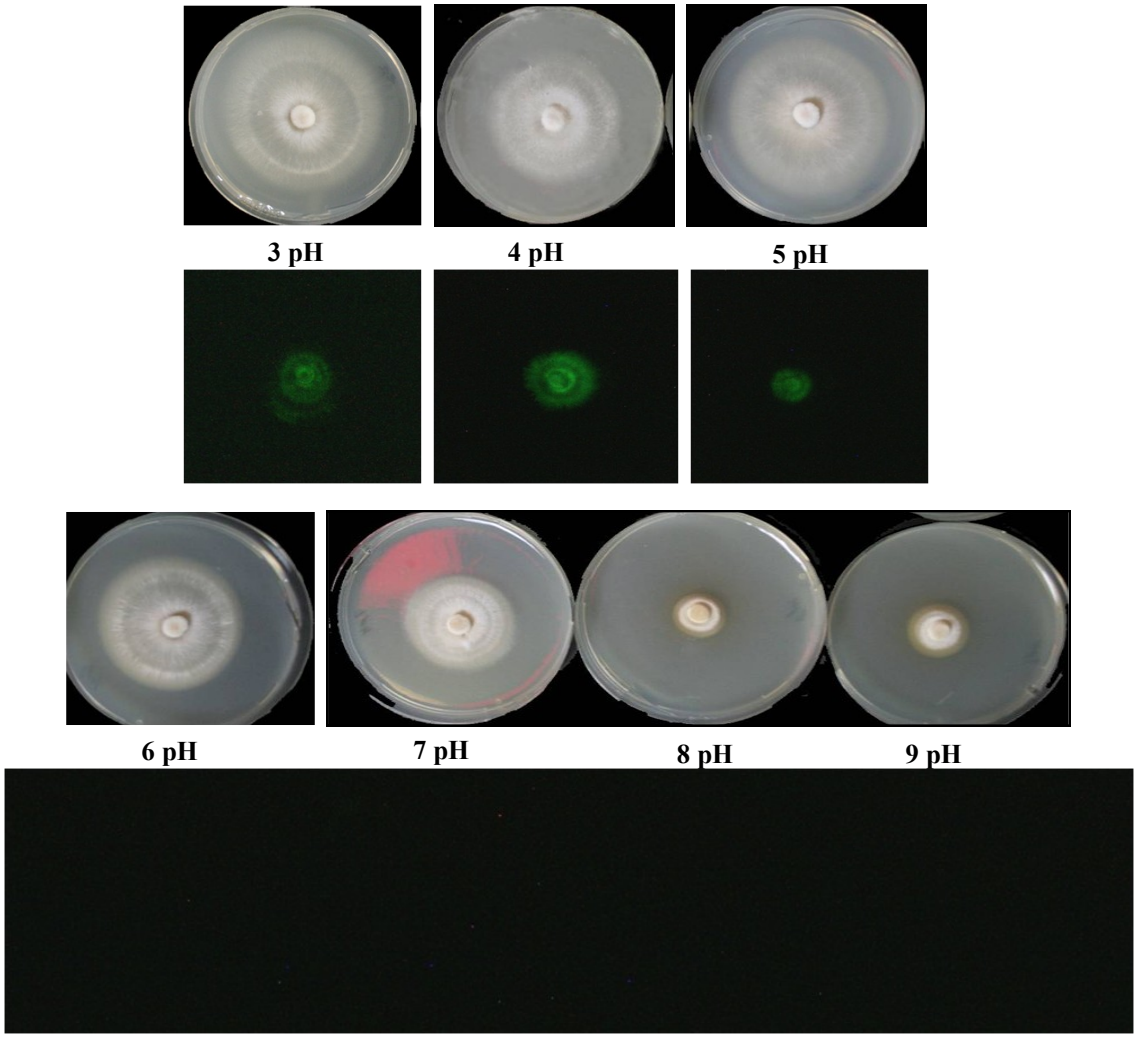

Plate No. 3 Luminescence (RLUs) of *Panellus stipticus* with selected H-ion concentration after 15 days of incubation at 24°C.

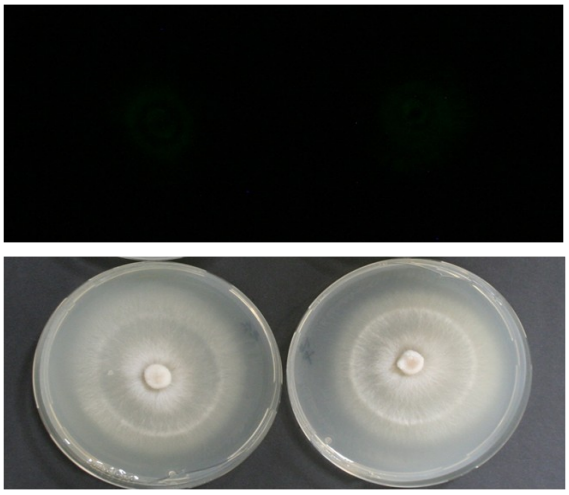

Plate. 4 Photo of Luminescence fungus *P. stipticus* during day light exposure and night conditions after 24 days of incubation.

IDP/Sen/75/04 dated 16.01 2006. The culture of *P. stipticus* was purchased from Lux Biotech. Ltd. U.K. The authors are thankful to Chairperson, Department of Botany for providing the necessary lab facilities and to UGC, (SAP, DRS-II) for the necessary equipment and infrastructure.

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