

# Effect of Color of Light Emitting Diodes on the Development of Mycelial Growth, Conidiation and UV Radiation in *Beauveria bassiana* (Balsamo) Vuillemin

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**Abstract:** The entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin is one of the most important biological control agents used in IPM programs and has many commercial products. Similar to other insect fungal pathogens, *B. bassiana* can produce conidia for dispersal, transmission and infection to other insect pests via a sexual reproduction. In the present study, mycelial growth, conidiation and UV resistance of *B. bassiana* under six colors of Light Emitting Diode (LED) conditions (red, blue, purple, green, yellow and white) is reported. Fungi cultured in dark chambers and exposure to LED light, *B. bassiana* hyphae grow continuously and did not differentiate in each treatment whereas conidia yield after exposure with different colors of LED light at 1, 3, 7, 14 and 24 h were significantly different. The results show that *B. bassiana* conidia yield were different significant, depending on color: red light induced the highest conidia yield after exposure for 1 h, while blue light had lowest conidia production, indicating that colors of LED light could stimulate conidiation of *B. bassiana*. All of the conidia were tested with UV tolerance, conidia of *B. bassiana* from red LED light was highly tolerant to UV radiation. The identification in this study of colors of LED light with relatively high conidia yield and UV tolerance will guide the selection of colors of LED light for researchers to develop an efficient *B. bassiana* as commercial product for biological control of insect pests in the future.

**Keywords:** Light Emitting Diode, *Beauveria bassiana*, UV tolerance, Conidiation

## 1. Introduction

Light energy is essential to all life on the earth. It is a crucial environmental signal that regulates the development and physiology of all organisms including fungi and entomopathogenic fungi. Light is one of many signals that fungi use to perceive and interact with their habitats [1-3]. Light emitting diodes (LEDs) have longer life and greater energy efficiency than another lamps, and LED light does not emit heat rays and UV radiations [4, 5]. Light regulates the growth, metabolism and reproduction of fungi and thus is important for the development of fruiting bodies, morphogenetic in *Hypsizygus marmoreus*, *Lentinula edodes*, *Pleurotus erygii*, *Cordyceps militaris* [6-8]. Therefore, the effects of LED light stimulated mycelial growth and conidiation of *Aspergillus ficuum*, *Metarhizium robertsii*, *Metarhizium anisopliae*, *Neurospora crassa*, *Coprinus stercorarius* and *Aspergillus nidulans* [9-14]. Like other insect fungal pathogens, *B. bassiana* is known as a fungal pathogen and has a high potential to infect a variety of insect pests including agricultural pests and insect vectors of human pathogens [15-18] and has been intensively investigated as an important bio-control agent in IPM programs around the world. *B. bassiana* usually produces asexual reproductive bodies, conidia, for dispersal, transmission and infection of host insects [19]. However, the mechanism of conidiation in *B. bassiana* remains poorly understood, which limits the improvement in conidia production and development of this organism as a bio-insecticide. In Thailand, there has been very little research conducted to investigate the effects of LED light on fungal cultivation. Adequate mass-production, LED light conditions of high quantity and quality conidia is

crucial to develop an efficient bio-insecticide and all these LED light-affected mechanisms may be important to protect conidia against UV radiation [20, 21]. The aim of this work is to demonstrate the effect of color of LED light on development of mycelial growth, conidiation and UV radiation in *B. bassiana*. The quality of light sources was carefully defined in this study to avoid any deviation in light source. The effect of light on the growth of fungi on media culture may also act as an index for mycelial growth and conidiation. Understanding the effect of light on mycelial growth and conidiation on plates may provide important information in the culture for bio-insecticide products. Examining the quantity and quality of conidia on plates would save time and reduce costs of media cultivation.

## 2. Materials and Methods

### 2.1 Strain and growth condition

*B. bassiana* isolate BCMU4 was obtained from Rajamangala University of Technology Lanna Collection of Entomopathogenic Fungal cultures, Lampang. BCMU4 was isolated originally from *Nilaparvata lugens* (Stål) (Brown plant hopper) [Hemiptera: Delphacidae] in Pitsanulok, Thailand. Stock cultures were maintained at 25±1°C in test tubes on slants of potato dextrose agar supplemented with 1 gL<sup>-1</sup> yeast extract (PDAY) adjusted to pH 6.9 for 15 days with a photoperiod of 12:12 h (Dark:Light). Petri dishes (90 mm in diameter) containing 15 mL MEA (malt extract 4% soybean peptone 1% and agar 1.5%) were inoculated in the center with a droplet of 5 µl conidia suspension at a concentration of 1×10<sup>8</sup> conidia/ml; incubated under dark

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condition at 25±1°C for 15 days. Plugs (5 mm in diameter) from the growing edge of a colony were then used to inoculate the center of 90-mm Petri dishes containing 20 ml fresh MEA. The plates were wrapped in aluminium foil and incubated at 25±1°C until the colonies were adaptable for LED stimulation.

## 2.2 Mycelial growth

The preliminary test was conducted using *B. bassiana* isolates BCMU4 exposed for 1, 3, 7, 14 and 24 h with six colors of LED light. Differences between the six colors of light and the effects of the seven exposure times on viability were assessed using an analysis of variance of a 3×7 factorial in a randomized block design. (the LEDs light Chamber could accommodate only 32 Plates at one time). Colonies grown under LED light were incubated at 25±1°C in LED a light chamber positioned 40 cm above the agar surface. The temperature of the culture was measured using HOBO ware. For LED light color, a piece of red, blue, purple, green, yellow, white light filters with transmission spectra of >300, 300-700, 280-400, >400, >425 nm, respectively. Four replicate dishes per exposure time were used in each trail. The experiments had two controls by being maintained in dark and fluorescent light conditions.

## 2.3 Conidiation yield

Colonies treated with different Photoinduction conditions were incubated for 21 days, and conidia were scraped from the agar surface and suspended in sterilized Tween 80 (0.05%). The hyphal debris was filtered through a sterilized polycarbonate membrane (25-mm diameter, 8-µm pore size, Whatman Nucleopore, Clifton, NJ, USA) to remove spore aggregates and the number of conidia was determined with a hemocytometer. Three replicate dishes per exposure time were used in each trial. The experiments had two controls by being kept in darkness and fluorescent light conditions. Variation in the conidial yield at a given experiment was differentiated among the treatments by one-way ANOVA and significance between treatments was tested with the LSD method.

## 2.4 Comparisons of conidia yield by six LED light colors: UV tolerance

To prepare inoculum for UV exposures, conidia suspensions selected from different LED light conditions, five agar plugs were removed from each plate at random places in the medium with a cork borer (5 mm diameter) and all five (total surface area ~90mm<sup>2</sup>) were place in 2 ml of sterile Tween 80 (0.05%). The conidia were suspended by vigorous vortexing and conidia were filtered through a sterilized polycarbonate membrane (25-mm diameter, 8-µm pore size, Whatman Nucleopore, Clifton, NJ, USA). Conidial concentrations were determined by hemocytometer counts and dilutions made with sterile Tween 80 (0.05%) for immediate use in the radiation studies. For each of the three trials, conidia obtained from MEA medium (40 µl, 1×10<sup>5</sup> conidia ml) were spread onto MEA (15 ml) plates (polystyrene 90×15 mm, Petriq), using a sterile glass spreader. Conidia were exposed to UV for 1, 2, 3 and 4 h. Four replicate dishes per exposure

time were irradiated in each trail. Control conidia were not irradiated but were placed in the chamber covered with an aluminium foil barrier. Germination was also observed with 400×magnification at 6 h from the exposure time. Conidia presenting a germ tube longer than the diameter of the conidia were considered to have germinated. A total of 300 conidia per treatment were evaluated. For each trial, relative percent of germination was computed as treatment/control×100% after each incubation time.

## 3. Results

### 3.1 Effects of LED light and exposure time on mycelial growth and conidiation of *B. bassiana*

To determine the optimum light exposure required for stimulating mycelial growth and conidiation, colonies of *B. bassiana* grown under total darkness for 48 h were exposed to different durations of LED light colors (red, blue, purple, green, yellow and white). One hr doses under LED light colors, *B. bassiana* exposed to continuous six colors of LED light percentage of mycelial growth were not significantly different from that under fluorescent light and darkness. Mycelial formed compact and flat colonies with prolific conidiophores bearing conidia, which were similar to those under total fluorescent light and darkness conditions (Figure 2). Conidia yields gradually increased with the increase of different colors of LED light. When the light pulse duration was prolonged from 1 to 24 h the conidia yields were significant different. As in Table 1 exposure of 1 h, the conidia production under fluorescent light condition (1.62×10<sup>9</sup> conidia per colony) was not significantly different from that under darkness (1.67×10<sup>9</sup> conidia per colony). The maximal conidia yield was obtained with red LED light (3.90×10<sup>9</sup> conidia per colony), while those under green, yellow, purple, white and blue light were significantly lower, with 1.91, 1.32, 1.23, 1.05 and 0.46×10<sup>9</sup> conidia per colony decrease compared to that under red light, respectively (F-test=significant at P<0.001; LSD=0.06). These results suggest that red light was most effective in stimulating conidiation. In addition, fewer conidia (0.46×10<sup>9</sup> conidia per colony) were detected from blue light-growth colonies, suggesting that LED light color significantly stimulated but was not essential for conidiation.

### 3.2 Comparisons of conidia produced under eight light condition with UV radiation

Conidia of *B. bassiana* produced under red LED were tolerance to UV radiation, and this level was higher than that of conidia raised under blue, purple, green, yellow, white, fluorescent light and dark (Figure 1). Growth, conidiogenesis and UV tolerance of fungus grown under different light conditions significantly changed both the conidiation produced under LED lights and the tolerance to UV radiation of *B. bassiana* conidia.

#### 4. Discussion

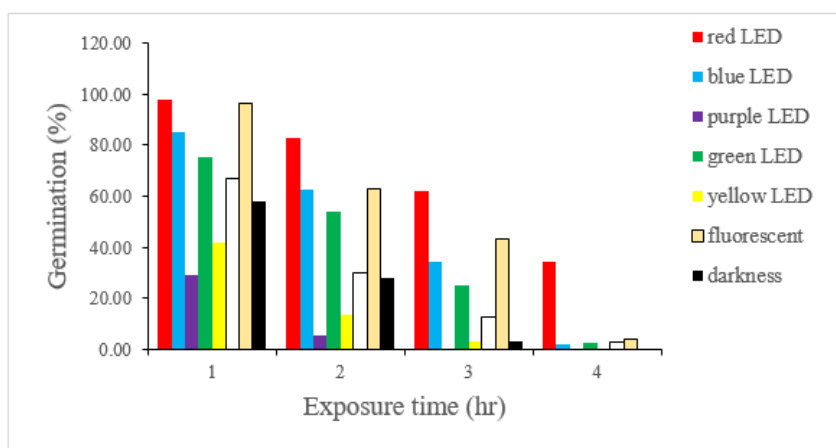
The effect of LED light to growth, development, conidiation, and fruiting body development of fungus are highly light dependent and induced by blue and red light and has been reported by Leatham and Stahmann [6-10, 12, 13, 22]. In our study, the results of conidiation showed that LED light of different colors and different times to exposure effect the quantity of *B. bassiana* conidial yield. For exposure to LED lights 1 h, red LED condition was found to be the most effective stimulator of conidiation of *B. bassiana* for maximum development of conidia. When exposed to blue, purple, green, yellow, white, fluorescent light and darkness condition the conidia was poor. Like, Lee et al [23] who studied in *Magnaporthe oryzae* fungus and reported that

asexual spore release is controlled by both blue and red light but in this study, blue light produced a minimum of conidia. Consistent with Röhrig et al [13] who reported that blue and purple light have the ability to reduce mycelial growth of *Aspergillus nidulans* fungus. Furthermore, *B. bassiana* conidia produce under red LED condition have the highest ability to tolerance UV radiation similar to *M. anisopliae* [11, 24]. Where understanding the role of LED light and time in conidiation will provide information to further clarify the mechanism of conidiation in *B. bassiana*.

**Table 1:** Quantification of *B. bassiana* conidial yield under exposure to different color of LED light and different time exposures

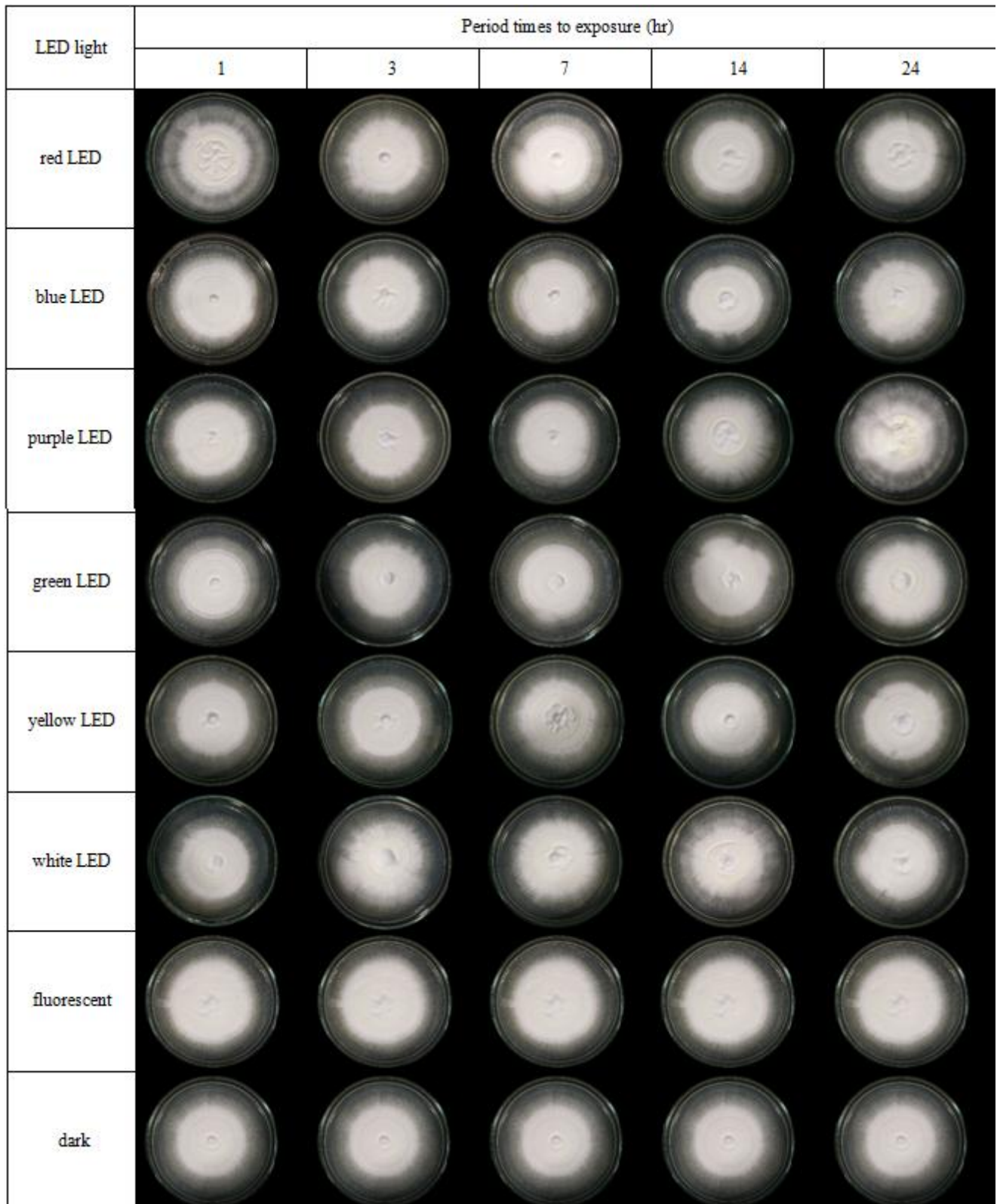
LED light	Conidial yield of <i>B. bassiana</i> at 21 days <sup>1</sup>				
	Period times to exposure (h)				
Red LED	3.83×10 <sup>9</sup> a <sup>2</sup>	2.00×10 <sup>9</sup> a	1.00×10 <sup>9</sup> c	1.33×10 <sup>9</sup> bc	1.00×10 <sup>9</sup> d
Blue LED	0.46×10 <sup>9</sup> f	0.54×10 <sup>9</sup> e	0.55×10 <sup>9</sup> e	1.12×10 <sup>9</sup> cd	1.90×10 <sup>9</sup> a
Purple LED	1.23×10 <sup>9</sup> d	1.79×10 <sup>9</sup> b	0.67×10 <sup>9</sup> e	1.51×10 <sup>9</sup> bc	0.49×10 <sup>9</sup> e
Green LED	1.91×10 <sup>9</sup> b	1.55×10 <sup>9</sup> c	1.05×10 <sup>9</sup> c	1.27×10 <sup>9</sup> bc	1.22×10 <sup>9</sup> c
Yellow LED	1.32×10 <sup>9</sup> d	1.03×10 <sup>9</sup> d	0.82×10 <sup>9</sup> d	0.85×10 <sup>9</sup> d	1.05×10 <sup>9</sup> cd
White LED	1.05×10 <sup>9</sup> e	1.72×10 <sup>9</sup> bc	1.78×10 <sup>9</sup> a	2.31×10 <sup>9</sup> a	0.97×10 <sup>9</sup> d
Fluorescent	1.62×10 <sup>9</sup> c	1.62×10 <sup>9</sup> bc	1.62×10 <sup>9</sup> b	1.62×10 <sup>9</sup> b	1.62×10 <sup>9</sup> b
Control Dark	1.67×10 <sup>9</sup> c	1.67×10 <sup>9</sup> bc	1.67×10 <sup>9</sup> ab	1.67×10 <sup>9</sup> b	1.67×10 <sup>9</sup> b
F-test	***	***	***	***	***
LSD <sub>0.05</sub>	0.06	0.09	0.07	0.21	0.10
CV (%)	4.35	7.59	7.11	16.59	10.01

\*\*\* = significant at P < 0.001; <sup>1</sup>average from 3 replication; <sup>2</sup>average follow by the same letter do not differ significantly at p=0.05 according to Least Significant Difference



**Figure1:** Germination rate of conidia irradiated at 28°C





**Figure 2:** Colony growth of *B. bassiana* under different color of LED lights and different time to exposure as indicated on MEA at 21 days after incubation

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