Effect of Culture Parameters of Light Intensity and Nutrients on Germination of *Alternaria Solani* Conidia

Mayukh Das, Subhadip Brahmachari, Amitava Saha, Surekha Kundu

Abstract: ***The necrotrophic pathogen Alternaria solani fsp. Lycopersici is an important pathogen for solanaceous crops and is cultured in laboratories for various purposes. The production of spores in culture under optimum conditions is very different from that occurring in the natural environment. Therefore it is important to optimize various culture conditions to understand the effect these conditions have on the spores of the fungus. Most studies focus on sporulation rather than spore germination and growth of the germ tubes. This study investigates the effect of different culture conditions such as light intensities, darkness and presence of nutrients on spore germination and germ tube length over a time course in A. solani. Here, we observed that the germination is delayed in dark in comparison to when incubation is done in presence of light. Light appears to affect the growth of germ tubes more significantly compared to germination frequency of the spores. The implication of the findings, in the context of germination of fungi in presence of nutrients is also discussed.***

Index Terms: Alternaria Solani, Light Intensity, Germination Frequency, Germ Tube Length, Culture Condition.

I. INTRODUCTION

Research in plant pathology involves the optimized culture of different pathogens specially filamentous fungi. Sporulation of filamentous fungi in culture and the germination of these spores are important aspects of fungal cultures due to frequent utilization of fungal spore to infect plants [1]. The necrotrophic pathogen *Alternaria solani* fsp. *lycopersici* is the causal organism of early blight and fruit rot disease of tomato. The pathogen causes infection on leaves, stem, petiole, twig and fruits as well as leads to the defoliation, drying of twigs and premature fruit drop which ultimately reduce the production. The spores or conidia are the specialized structures responsible for the fungal dispersal and environmental persistence in *A. solani* [2]. Therefore spore suspensions of *A. solani* are commonly used as inoculums and subsequently the disease progression in terms of spore germination and germ tube length on host surface are studied. Filamentous fungi like *A. solani* is an extremely versatile organism, which can grow under different environmental parameters [3]. Fresh spores are obtained after mycelium had been grown on semi-synthetic media like potato dextrose agar (PDA) for about ten to fourteen days [4]. To study host pathogen interaction using *A. solani*, the pre-requitsite is to find the optimum culture conditions.

Environmental conditions like temperature, light intensity, nutrition, pH and oxygen concentration affect the growth and development of the pathogen [5], [6]. Production of spores in the laboratory under optimum conditions is very different from that occurring in the natural environment [7]. Therefore it is important to optimize various culture conditions to understand the effect these have on the spores of the fungus. In the present report the effect of different culture conditions viz. effect of absence of light (dark condition) and light intensity have been studied in two different media conditions i.e. in normal nutrient medium (PDA) and plain Agar medium where nutrients are completely absent. The parameters studied were the rate of germination of spore or conidia and also the frequency of germination of conidia.

II. MATERIALS AND METHODS

A. Fungal Material:

The tomato early blight fungi *Alternaria solani* fsp. *Lycopersici* (Indian Type Culture Collection, Indian Agricultural Research Institute, ITCC No. 4632) was used for this study. The culture was maintained in pure form and stored in 4˚C. The fungal cultures were sub-cultured on potato dextrose agar (PDA) medium (HIMEDIA) and maintained in 28˚C. The spores or conidia were observed under compound microscope (Leica).

B. Preparation of Spore Suspension

Two week old *A. solani* culture was used for collection of spores. The black masses of spores were scraped out from the petridish and suspended in 20ml sterile distilled water in a 100ml conical flask. The spore suspension was filtered through tri-folded cheesecloth and the number of spores per unit volume was counted using a haemocytometer under a compound microscope (Leica). The final concentration of spore was adjusted to 1×10^5 spores/ml.

C. Preparation of Slides

First PDA and 2% water agar were prepared and sterilized. Under aseptic conditions, sterilized glass slides were placed.

Revised Version Manuscript Received on 21 October, 2018.

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The glass slides were laid with PDA and agar media. Finally, the spore suspensions were spread onto the media. For both the light and dark conditions, the above media were used. The incubation was done in different conditions for each experiment.

D. Experimental Set Up under Dark Condition

The glass slides with PDA and agar media were used separately. The 50µl spore suspensions of *A. solani* (of concentration 1×10^5 spores/ml) were spread on the media surface of the slides. These were kept in the dark (temperature=28˚C). Spore germination frequency as well as the lengths of germ tubes were observed under compound microscope (Leica, magnification 40×) and measured at different time periods of 0h, 2h, 4h, 6h, 8h and 24h.

E. Experimental set up at Three Different Light Intensities:

For the light condition, media coated slides along with 50µl of spore suspension of *A. solani* were prepared and incubated under three different light intensities viz. 5 lux (LLI= Low Light Intensity), 20 lux (MLI= Medium Light Intensity) and 120 lux (HLI= High Light Intensity). Observations were made at different time points of 0h, 2h, 4h, 6h, 8h and 24h and the spore germination frequencies along with germ tube lengths were observed under compound microscope (Leica, magnification 40×) and calculated.

III. RESULTS AND DISCUSSION

The present study investigates the effect of light intensity and dark incubation as well as nutrient conditions on fungal behavior in terms of spore germination frequency and germ tube length in *Alternaria solani*. The primary goal of this study was to evaluate the different conditions to get optimum conditions of culture of this fungus for various purposes. Another objective was to see if light and nutrients promote or inhibit spore germination and germ tube growth.

Observations of spore germination frequency as well as the germ tube length of *A. solani* at different light intensities (dark, 5 lux, 20 lux and 120 lux) in absence and presence of nutrients (plain agar and PDA) were taken into consideration to address the specific objectives of the present work.

A. Effect of Light Intensity on Spore Germination

The observation showed that spore germination frequency was influenced by different light intensities (0 lux i.e. dark, 5 lux, 20 lux and 120 lux) (**Figure 1 and 2**). Higher frequencies of spore germination were achieved in higher light intensity in comparison to that achieved in lower intensity on both the media used viz. agar (**Figure 1C**) and PDA (**Figure 2C**). Along with spore germination frequency, the length of the generated germ tube seemed to be under direct influence of light intensity. Germ tube lengths were longer with increasing light intensities (**1-A,B** & **2-A,B**) (**Table 1, Table 2**). Analysis of the obtained result showed a distinct trend of increase in both spore germination frequency and germ tube length with increasing light and this trend is similar for both the minimal media (agar) as well as basal fungal media (PDA). However spore germination frequency and germ tube length were lower in absence of nutrient as seen in agar medium compared to when nutrients were provided (as in PDA) (**Figure 3A and B**). The incubation time needed to achieve approximately 60% spore germination rate in Agar medium incubated in different light intensities and darkness was 24 hours, whereas similar germination rate (60%) was recorded in PDA medium after only 2 hours and 4 hours of light and dark conditions respectively. Many researchers have reported the growth and germination frequency of different fungi in different culture media resulting in the occurrence of maximum growth in PDA than other media [8]. Similar result was obtained in this study also where the germination rate is high in PDA medium compared to Agar medium in different light conditions (darkness and three different light intensities).

There are several reports regarding the effects of light on mycelial growth and sporulation of fungi [9] but there are very few reports available depicting the role of different light intensity on spore germination process. Although fungi are unable to perform photosynthesis hence do not require light for this purpose, but the importance of light in mycelial growth, spore germination has already been established by previous studies [5], [10]. The effect of light on fungi is species-specific that can either promote or inhibit the growth of mycelium and other structures [3] but nevertheless it is a major parameter in the spore germination process in fungi. In the present study, the presence of light during culture, increased spore germination and germ tube length but light had more significant effect on the germ tube length than germination frequency (**Table 1 and Table 2**). Although light is essential factor for growth [10], excess intensity impacts negatively on it that might results in growth inhibition in previous report [3]. In this study, it was interesting to note that the germination of *A. solani* spores was not inhibited by direct exposure of light.

Table 1: Spore Germination Frequency and Length of Germ Tube of *Alternaria solani* on Agar Media under Different Light Intensity

hpi	Agar							
	Germination frequency (%)				Germ tube length (µm)			
	Dark	5 lux	20 lux	120 lux	Dark	5 lux	20 lux	120 lux
0h	0	0	0	0	0	0	0	0
2hrs	24.33 ±7.21	34.17 ±1.53	41.31 ±1.26	43.62 ±2.08	6.6 ±2.14	16.54 ±3.02	19.68 ±0.74	19.68 ±1.53
4hrs	30 ±4.53	38.83 ±0.52	43.47 ±0.76	50.8 ±1.51	9.84 ±1.51	17.54 ±3.00	19.68 ±1.62	19.68 ±1.06
6hrs	44.8 ±4.81	46.27 ±0.5	52.83 ±0.97	56.1 ±1.83	12.92 ±1.5	19.81 ±2.3	26.37 ±1.93	32.67 ±2.62
8hrs	52.2 ±5.09	54.5 ±1.53	58.59 ±1.04	61.5 ±1.44	16.7 ±1.31	22.97 ±2.8	29.52 ±1.86	36.20 ±1.15
24hr	60.5 ±2.5	61.89 ±1.39	64.22 ±0.59	68.67 ±0.59	22.8 ±2.15	26.84 ±0.51	39.37 ±1.85	47.24 ±1.37

Table 2: Spore Germination Frequency and Length of Germ Tube of *Alternaria solani* on Potato Dextrose Agar (PDA) Media under Different Light Intensity

hpi	PDA							
	Germination frequency (%)				Germ tube length (µm)			
	Dark	5 lux	20 lux	120 lux	Dark	5 lux	20 lux	120 lux
0h	0	0	0	0	0	0	0	0
2hrs	44.53 ±7.51	55.67 ±3.82	57.83 ±1.25	62.67 ±4.05	9.8 ±1.52	16.54 ±1.81	22.6 ±1.65	33.61 ±0.88
4hrs	65.94 ±8.89	70.92 ±1.26	74.17 ±2.16	76.18 ±1.31	12.79 ±2.24	20.07 ±0.92	26.12 ±1.56	35.9 ±1.71
6hrs	74.55 ±7.22	78.5 ±2.89	81.21 ±1.89	85.22 ±1.49	12.99 ±2.11	21.65 ±2.17	40.75 ±2.82	66.16 ±0.76
8hrs	81.44 ±5.2	82.71 ±2.29	86.17 ±0.71	89.9 ±0.87	19.69 ±1.52	26.38 ±1.27	46.35 ±0.96	75.32 ±1.26
24hr	88.17 ±1.32	88.89 ±0.51	92.44 ±0.84	94.94 ±1.04	22.92 ±2.15	27.56 ±0.49	51.28 ±1.91	97.7 ±2.08

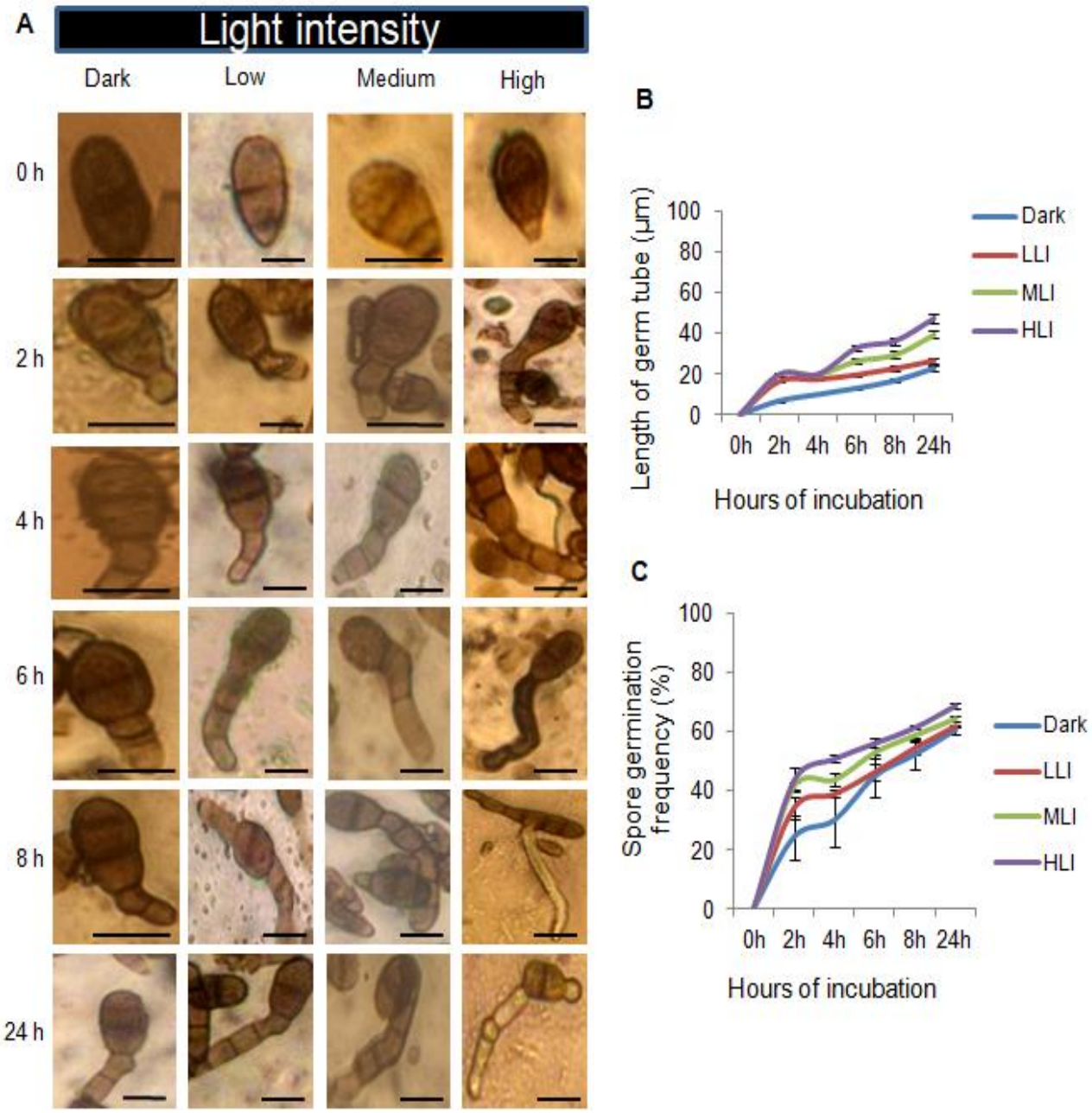

Figure 1: Differential State of Spore Germination of *Alternaria solani* in Absence of Nutrients under Different Light Intensities (dark: 0 lux, low: 5 lux, Medium: 20 lux and High: 120 lux) at Different Time Points on Plain Agar Medium. (A) Microscopic Images of Spore Germination (B) Length of Germ Tube, (C) Frequency of Spore Germination; LLI= Low Light Intensity, MLI= Medium Light Intensity & HLI= High Light Intensity; [Bar = 100µM].

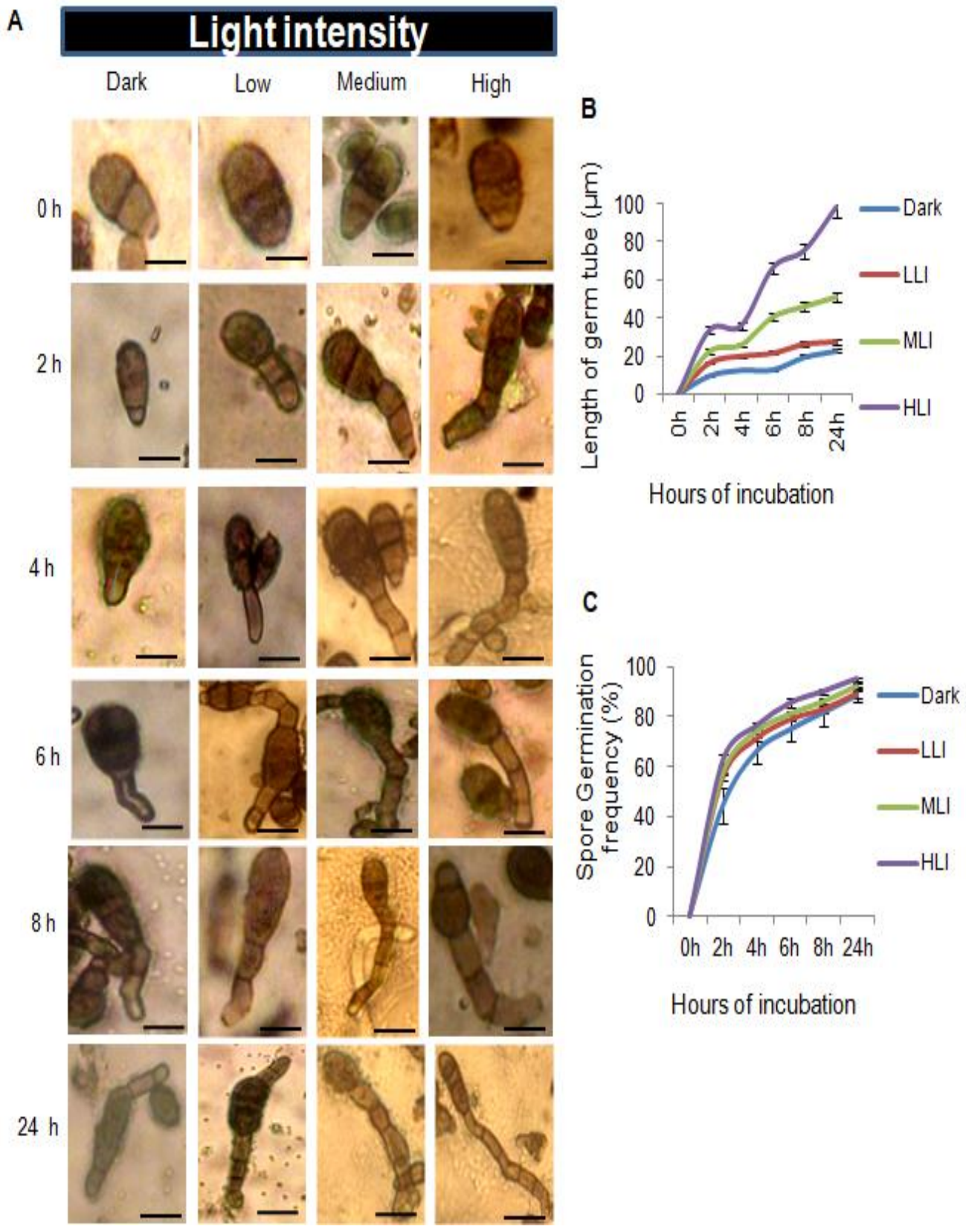

Figure 2: Differential State of Spore Germination of *Alternaria Solani* in Nutrient Medium (PDA) under Different Light Intensities (Dark: 0 lux, low: 5 lux, medium: 20 lux and high: 120 lux) at Different Time Points. (A) Microscopic Images of Germinating Spores (B) Comparison of Lengths of Germ Tubes Over a Time Course From 0-24 Hours in Different Light Intensities, (C) Frequency of Spore Germination Over a Time Course From 0-24 Hours in Different Light Intensities; LLI= Low Light Intensity, MLI= Medium Light Intensity & HLI= High Light Intensity; [Bar = 100µM].

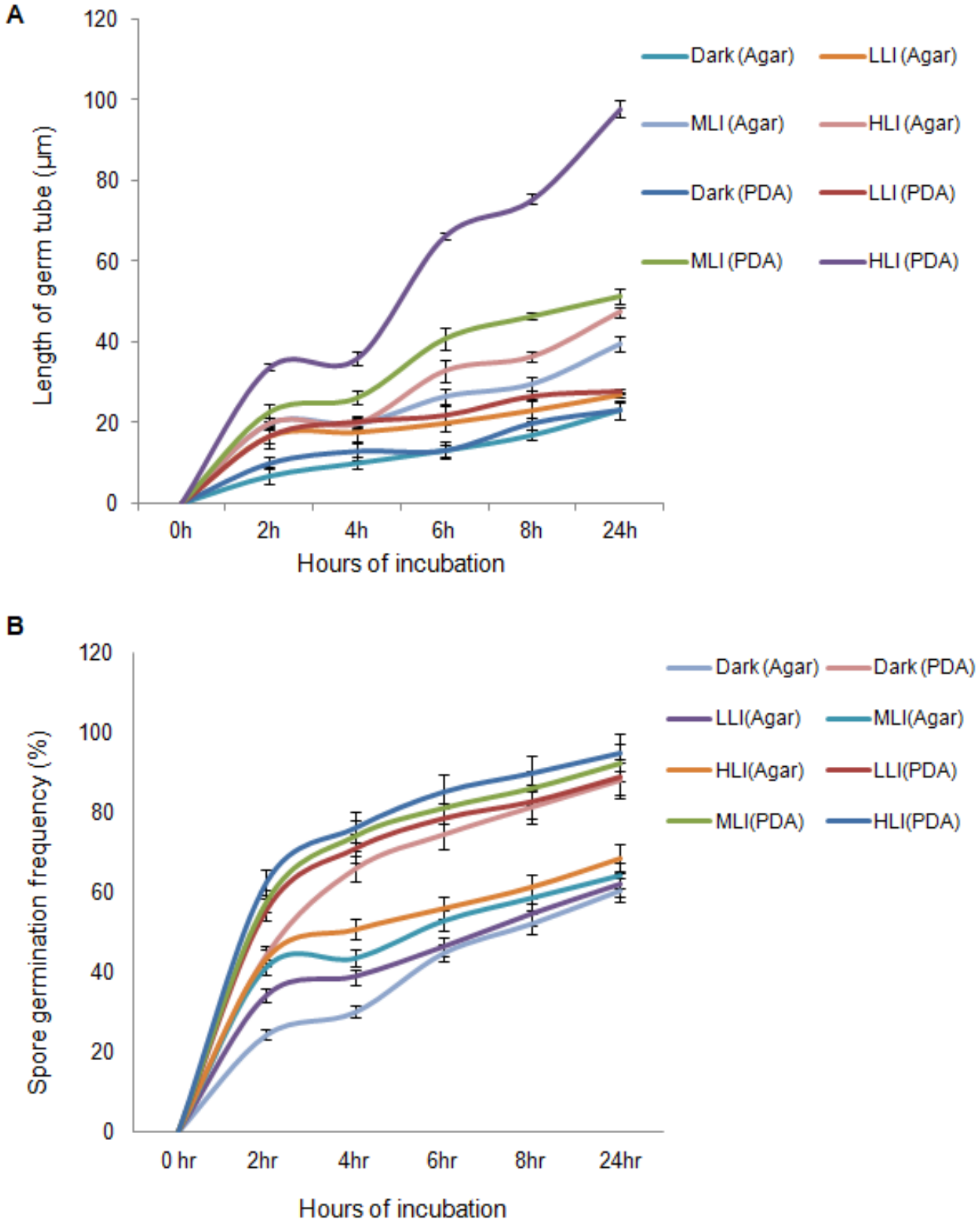

Figure 3: Comparison of Differential Rate of *Alternaria solani* Spore Germination Frequency (%) and Germ Tube Length (µm) on Agar and PDA Media in Presence Different Light Conditions i.e. dark (0 lux), low (5 lux), medium (20 lux) and high (120 lux). (A) Comparative Graphical Representation of Germ Tube Length in Agar and PDA Media. (B) Comparative Graphical Representation of Spore Germination Frequency (%) in Agar and PDA Media.

ACKNOWLEDGMENT

This work was partially supported by DBT and UGC CAS, Govt. of India. The authors are thankful to Department of Botany, University of Calcutta for providing necessary research facilities. The authors acknowledge Indian Agricultural Research Institute, Pusa, New Delhi for identifying the fungal material. The authors declare that there is no conflict of interest.

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