African Journal of Biotechnology Vol. 10(10), pp. 1863-1872, 7 March, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1967
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Influence of culturing conditions on growth and sporulation of *Drechslera hawaiiensis*, the foliar blight pathogen of *Marsilea minuta* L.

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Accepted 20 January, 2011

***Drechslera hawaiiensis* is responsible for foliar blight disease in *Marsilea minuta* L., a common weed of rice. Experiments were conducted for the determination of nutritional and climatic requirements of this pathogen. Five culture media viz. malt extract agar, potato dextrose agar, Richard's agar, water agar and Czapek Dox agar were used. Potato dextrose agar was found to be the best medium for culturing *D. hawaiiensis*. Sporulation of *D. hawaiiensis* was the best on potato dextrose agar medium followed by malt extract agar at 20 to 30°C. All the three pH levels viz. 6, 7 and 8 were found suitable for fungal growth and sporulation. However, the suitability of a particular pH was associated with temperature, culture medium and photoperiod. Both growth and sporulation were affected by photoperiod to which fungal colonies were exposed during incubation. In general, the effect of light on sporulation was more pronounced than the effect on fungal vegetative growth. On water agar and Richard's agar media, continuous darkness enhanced sporulation. Conversely, on potato dextrose agar and malt extract agar media, 12 h photoperiod was found to be the most suitable for abundant sporulation as compared to continuous dark or continuous light treatments. On Czapek dox agar medium, both continuous darkness and 12 h photoperiod were found to be equally effective for better sporulation. Presently, the interaction of temperature and light had a marked effect on sporulation in *D. hawaiiensis.* Under complete darkness, 25°C and under 12 h photoperiod, both 25 and 30°C were found to be most suitable for best sporulation in this species.**

Keywords: *Drechslera hawaiiensis*, growth media, light, *Marsilea minuta,* pH, temperature.

INTRODUCTION

Rice (*Oryza sativa* L.) is a major source of food for more than half of the world population. Only few other crops have as high nutritional value as that of rice. Carbohydrates in the form of rice starch form the largest nutrient group. In addition, vitamins, amino acids and minerals are also present in considerable quantities (Anonymous, 2003). Intensification of weeds is one of the reasons amongst biotic factors for the low yield of rice crop. Agricultural experts have experimentally assessed 20 to 65% losses in rice production due to weeds infestation (Alam, 2003). *Marsilea minuta* is a very common weed of rice in Pakistan (Rabbani and Bajwa, 2001). This weed is responsible for up to 20% reduction in yield losses in rice (Rabbani et al., 2010). Chemical method of weed control is the most popular among the farmers, because of its effectiveness. However, heavy reliance on herbicides in agriculture has increased the risk of environmental and food products contamination (Mancini et al., 2008). The increasing concern in public against excessive use of synthetic agrochemicals has led researchers to find alternative environmental friendly strategies for pest management which are based on natural products (Cuthbertson and Murchie, 2005; Javaid, 2010).

The use of native fungal pathogens as biological control agents is an alternative or complimentary tactic to reduce herbicide inputs (Kadir and Charudattan, 2000). In the recent, much work has been carried out on the use of indigenous fungal plant pathogens as biological agents for weed control (Siddiqui et al., 2009, 2010). During field survey of different regions of Punjab, Pakistan, *M. minuta*

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L. was found to be infected with foliar blight. *Drechslera hawaiiensis* was found to be associated with the diseased plants. Pathogenecity of the isolated fungus was confirmed through back inoculations onto host plant (Bajwa and Rabbani, 2000). The success of mycoherbicide depends on the best growth of the fungus on synthetic media, which facilitates the mass production of infective stage of fungus, either spores or vegetative mycelium. It is essential to have a clear understanding of the conditions which influence growth and sporulation of the fungus. The present study was conducted to have an insight into various factors affecting growth and sporulation of the *D. hawaiiensis.* The candidate agent was cultured on five media and subjected to various regimes of pH, temperature and light to evaluate the best suitable interactive conditions and to know the epidemiology of the pathogen. Findings of this study will contribute towards the knowledge on fungal cultural characteristics which will be an aid towards development of a mycoherbicide for the control of *M. minuta*.

MATERIALS AND METHODS

M. minuta plants suffering from blight were collected from rice field of University of Punjab, Lahore, Pakistan and brought to the laboratory in sterilized polythene bags. The diseased parts of leaves were cut into small fragments with the help of sterile scalpel, surface sterilized with 1% sodium hypochlorite, rinsed thrice with sterile water and inoculated on potato dextrose agar (PDA) and malt extract agar (MEA) plates in triplicate and incubated at room temperature. The fungus colonies that appeared on the media were isolated and identified as *D. hawaiiensis* with reference to literature (Domsch et al., 1980; Mukerji and Bhasin, 1986).

Five culture media viz. water agar, malt extract agar, potato dextrose agar, Czapek dox agar and Richard's agar were selected for culturing of *D. hawaiiensis*. Ingredients of each medium were weighed and mixed in 1000 ml of distilled water. The media were sterilized by autoclaving at 121°C for 20 min. Twenty milliliters of each sterilized medium was poured in 9 cm diameter sterilized Petri plates and allowed to solidify at room temperature.

A 5×3×3×3 factorial experiment with 5 growth media, 3 pH levels, 3 photoperiods and 3 temperature levels was designed in a completely randomized manner. For each of the five media, three pH levels viz. 6, 7 and 8; three photoperiods viz. 0, 12 and 24 hours; and three temperatures viz. 20, 25 and 30°C were employed in all possible combinations. Each treatment was replicated thrice.

A 0.5 cm diameter disc, cut with a sterilized cork borer from a 15 days old culture of *D. hawaiiensis,* was transferred in the center of media plates. Plates were incubated in growth chambers for 8 days under different conditions of light and temperature. The assessment of radial growth of the colonies of *D. hawaiiensis* was done after 8 days of fungal growth. Growth rate of the colony was determined by dividing the diameter of the colony with the total number of days.

The sporulation was determined by harvesting the conidia from the surface of the eight days old colonies of *D. hawaiiensis* by flooding each plate with 20 ml of sterilized distilled water and scratching the agar surface with the help of rubber spatula. The resulting suspension was filtered through muslin cloth and concentration of the spores was measured with the help of haemacytometer. Total number of spores on the colony and number of spores per unit area were calculated.

Data were subjected to analysis of variance (ANOVA), and means were separated using the Duncan's multiple range (DMR) test at 5% level of significance (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Effect of culture media on growth and sporulation of *D. hawaiiensis*

Considerable fungal vegetative growth was observed on all the five growth media (Figures 1 to 5). However, potato dextrose agar was found to be the best medium for culturing *D. hawaiiensis* (Figure 1). Generally, good growth of *D. hawaiiensis* on all the five growth media indicates that the species has ability to utilize a wide range of carbon sources and other nutrients. In contrast to the vegetative growth, sporulation of *D. hawaiiensis* was greatly affected by nutrient media. Sporulation was best on potato dextrose agar medium followed by malt extract agar at 20 to 30°C. Poorest sporulation was recorded on water agar medium (Tables 1 to 5). The least sporulation may be attributed to the absence of suitable carbon source and lack of other nutrients (Ilyas et al., 1995).

Effect of temperature on growth and sporulation of *D. hawaiiensis*

The present study reveals the suitability of 25 and 30°C for growth and sporulation on different media employed. However, these temperature optima for sporulation at different media were greatly influenced by the pH of the media and photoperiods to which the fungal cultures were exposed. Malt extract agar and Richard's agar, 25°C (Figure 2); water agar and Czapek Dox agar, 30°C (Figures 1 and 3); and potato dextrose agar, 20 to 30°C (Figure 4) were found most suitable for growth. Variation in fungal growth due to change in temperature has also been reported by other workers (Pardo et al., 2005; Meier et al., 2010). The most suitable temperature for sporulation on water agar and Czapek Dox agar was 25°C (Tables 1 and 3); on malt extract agar, 30°C (Table 2) and on potato dextrose agar and Richard's agar was 20 and 30°C (Tables 4 and 5). According to Cochrane (1958), temperature range permitting reproduction is usually narrower than that permitting growth. Earlier, Leach (1979) has reported variations in optimum temperature requirements within the same species for light induced sporulation.

Effect of pH on growth and sporulation of *D. hawaiiensis*

In the present study, all the three pH levels viz. 6, 7 and 8

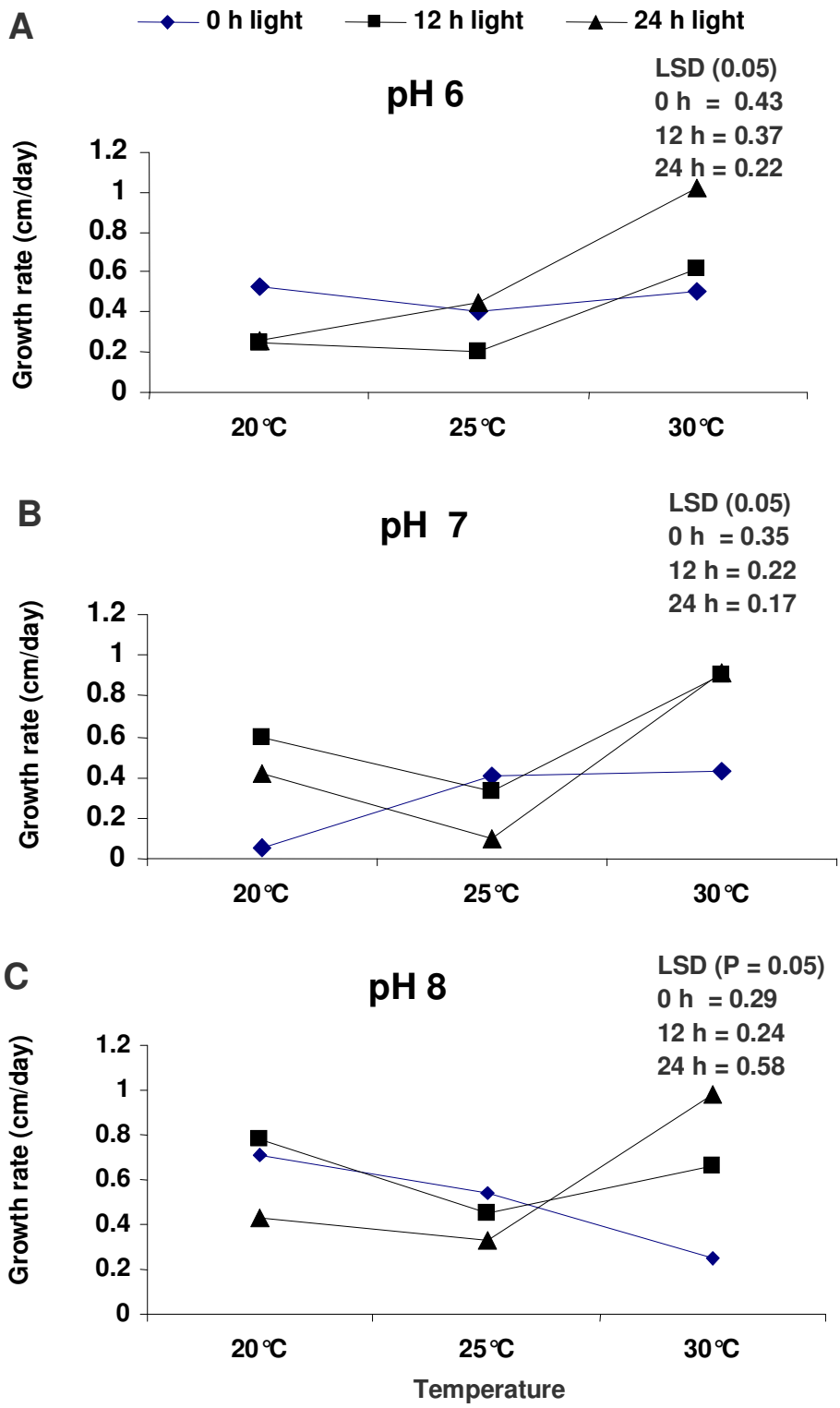

Figure 1. Growth rate of D. hawaiinsis on water agar medium under different conditions of light, pH and temperature.

were found suitable for fungal growth. However, the suitability of a particular pH was associated with temperature, culture medium and photoperiod. Any particular set of temperature and light conditions equally suitable for best fungal growth at a specific pH level on different growth media was not evidenced (Figures 1 to 5). The

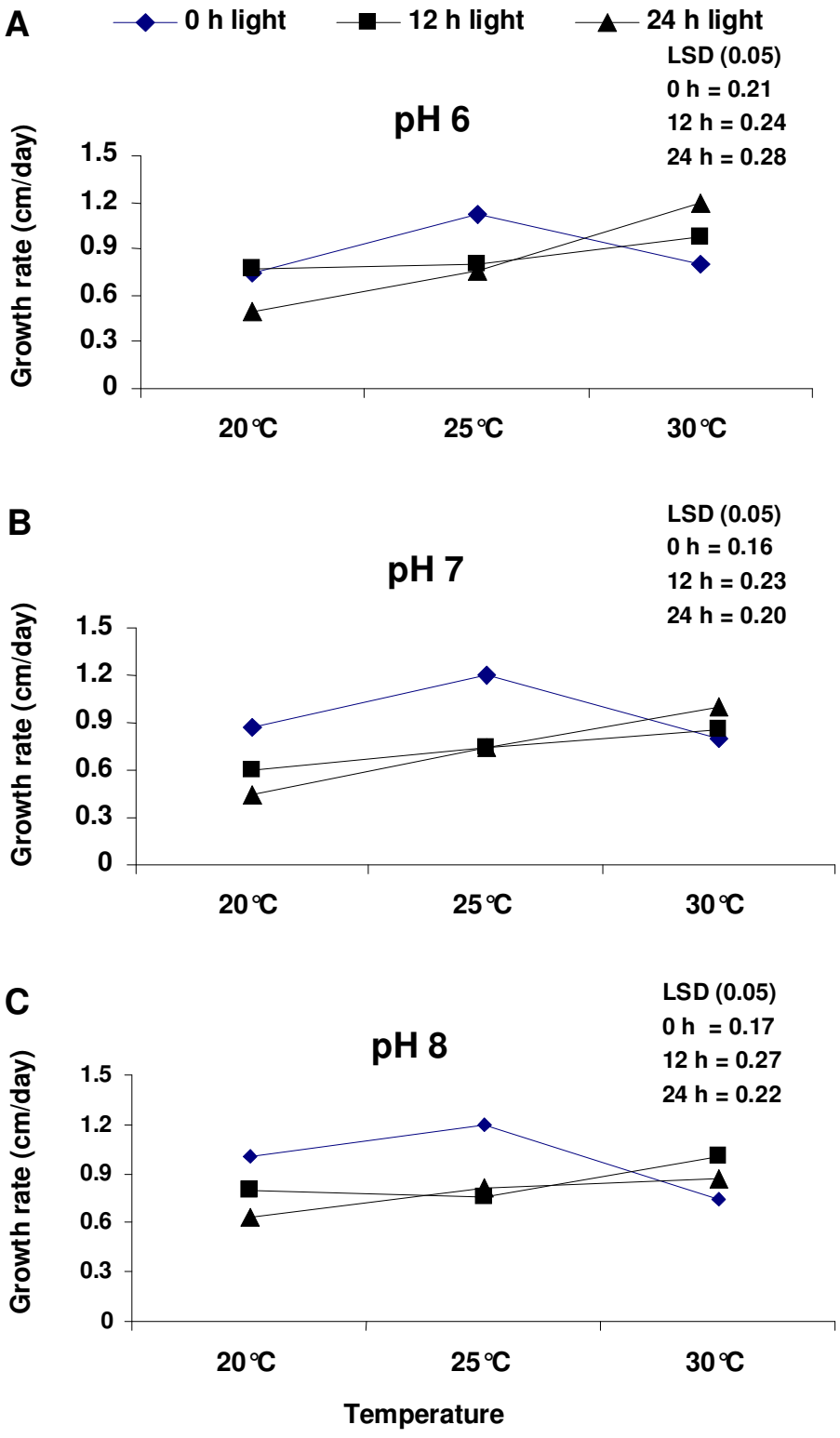

Figure 2. Growth rate of D. hawaiinsis on malt extract agar medium under different conditions of light, pH and temperature.

response of sporulation to pH was similar to that of vegetative growth of the fungus (Tables 1 to 5). Most fungi are known to tolerate a wide range of pH of the medium (Rousk et al., 2010). Inhibition of growth is usually

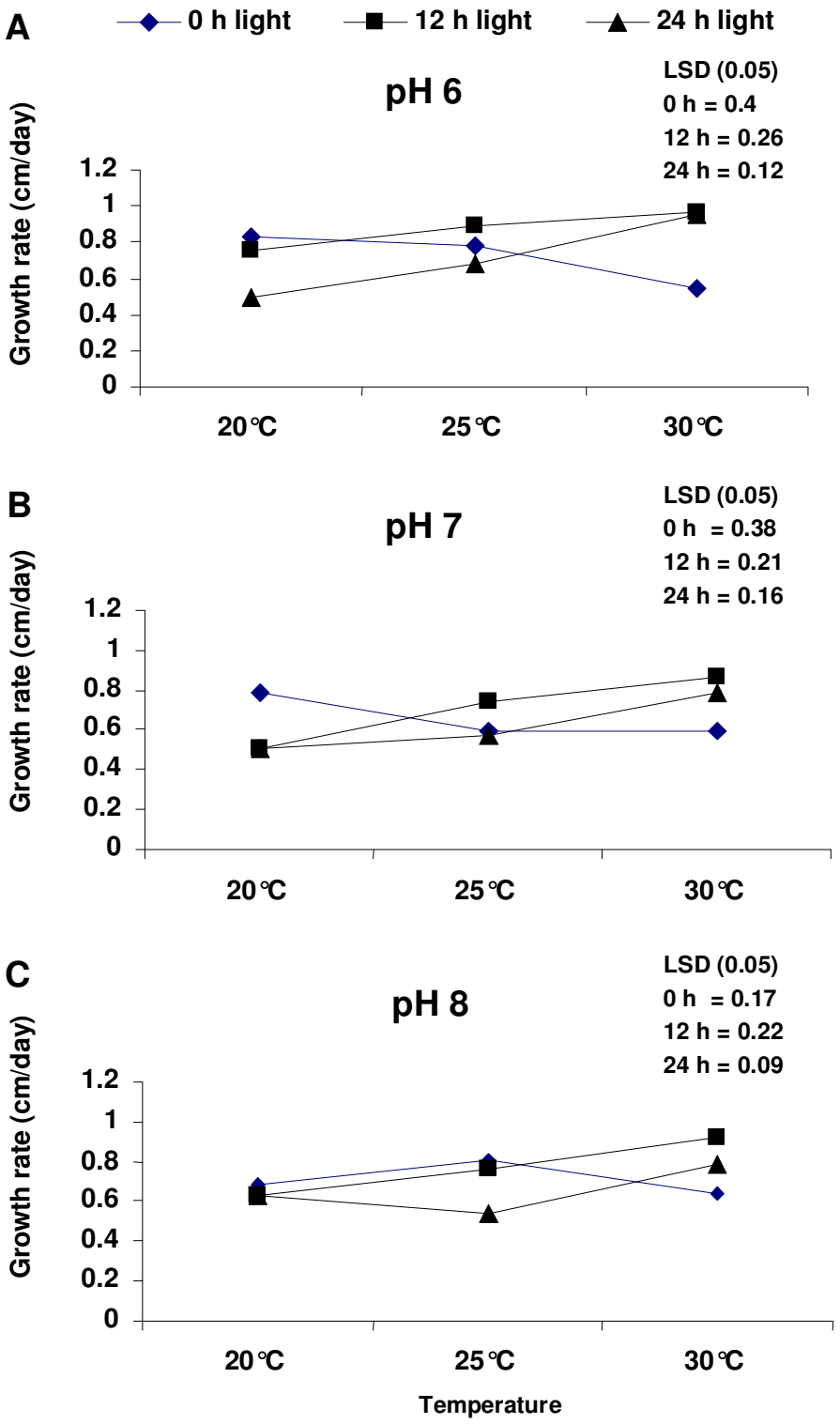

Figure 3. Growth rate of D. hawaiinsis on Czapek Dox agar medium under different conditions of light, pH and temperature.

defined at the limits of this range. Kumar and Grover (1967) found that pH for optimum growth and sporulation in *Lophotrichus ampullus* varied considerably. Generally, fungi grow best at neutral pH 7 or slightly on the acidic side of the neutral. There are, however, exceptions to these generalizations (Madan and Thind, 1998).

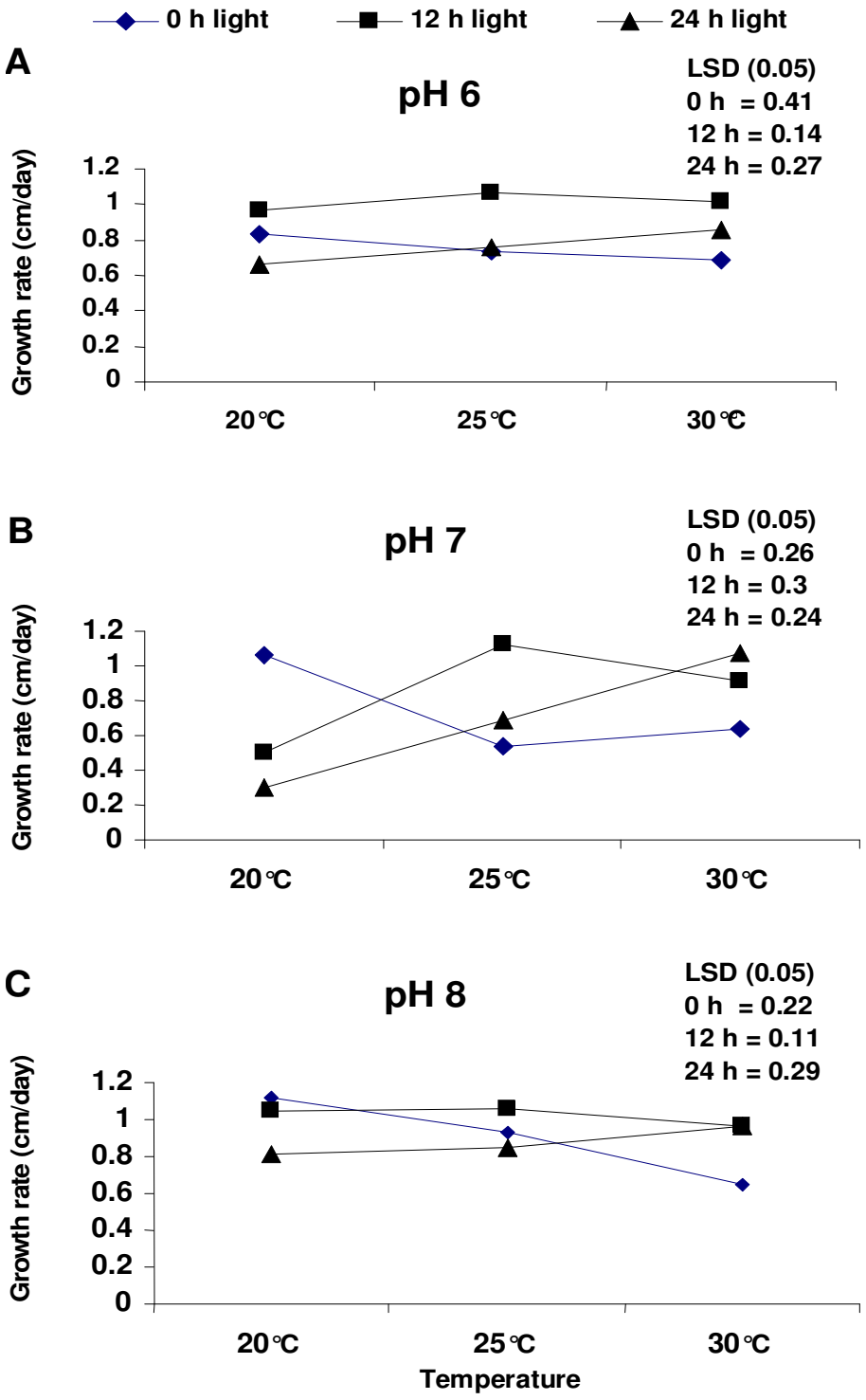

Figure 4. Growth rate of D. hawaiinsis on potato dextrose agar medium under different conditions of light, pH and temperature.

Effect of photoperiod on growth and sporulation of *D. hawaiiensis*

Both growth and sporulation were affected by photoperiod to which fungal colonies were exposed during incubation period. However, no generalized growth pattern was exhibited with reference to light on different growth media. A variable response of light was observed

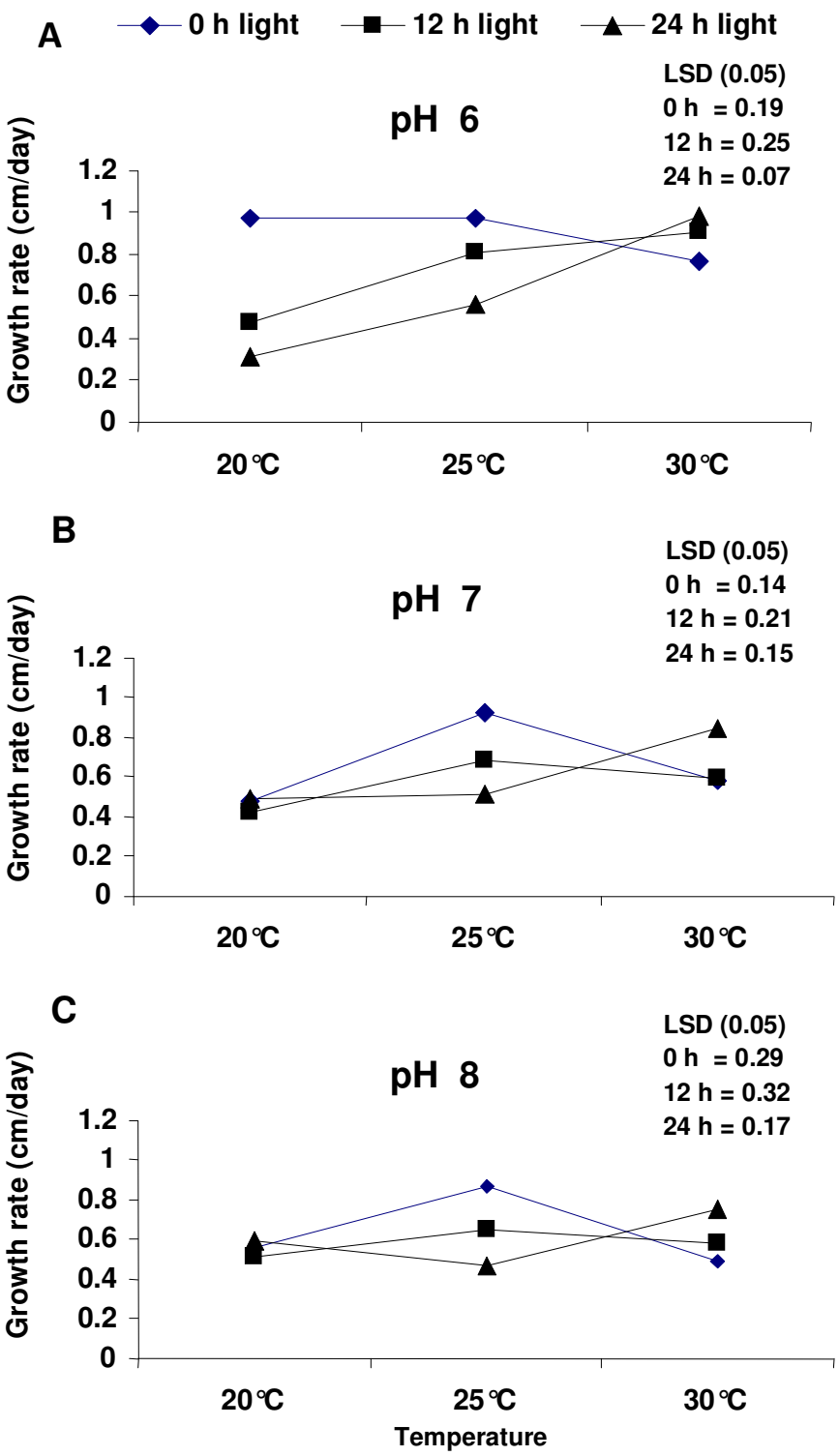

Figure 5. Growth rate of D. hawaiinsis on Richard's agar medium under different conditions of light, pH and temperature.

among specific temperature and pH regimes on different growth media. In general, the effect of light on the pattern of sporulation was more pronounced than the effect on fungal growth. On water agar and Richard's agar media, where sporulation was poorest, continuous darkness enhanced sporulation (Tables 1 and 5). Conversely, on

Table 1. Effect of light, temperature and pH on sporulation in *D. hawaiiensis* on water agar medium.

Parameter	Number of spores (cm^{-2})			Total number of spores on the colony		
	20°C	25°C	30°C	20°C	25°C	30°C
Complete darkness						
pH 6	5.0×10^1	2.4×10^2	0.0×10^0	1.9×10^3	4.7×10^2	0.0×10^0
pH 7	3.4×10^2	3.0×10^2	3.0×10^1	1.3×10^2	5.7×10^3	4.0×10^2
pH 8	1.3×10^1	1.8×10^2	7.1×10^1	5.3×10^2	6.1×10^3	1.3×10^2
12 h photoperiod						
pH 6	5.0×10^2	2.2×10^2	1.7×10^1	4.3×10^3	4.0×10^2	7.3×10^2
pH 7	2.7×10^2	2.2×10^1	6.0×10^0	8.3×10^2	1.3×10^2	4.7×10^2
pH 8	9.0×10^0	1.0×10^1	6.2×10^1	5.7×10^2	2.7×10^2	2.8×10^3
24 h photoperiod						
pH 6	2.4×10^2	3.2 ×10'	2.0×10^2	1.8×10^3	6.7×10^2	2×10^2
pH 7	3.9×10^1	1.8×10^1	5.0×10^2	8.0×10^2	6.0 × 100	4.7×10^2
pH 8	2.9×10^2	5.9×10^2	1.3×10^1	4.2×10^3	7.4×10^3	1.3×10^2

Table 2. Effect of light, temperature and pH on sporulation in *D. hawaiiensis* on malt extract agar medium.

Parameter	Number of spores (cm^{-2})			Total number of spores on the colony		
	20°C	25°C	30°C	20°C	25°C	30°C
Complete darkness						
pH 6	4.4×10^1	7.3×10^1	1.1×10^1	2.5×10^3	6.8×10^3	6.0×10^2
pH 7	6.5×10^1	3.6×10^1	3.3×10^1	4.5×10^3	3.9×10^3	1.9×10^3
pH 8	6.3×10^1	1.2×10^1	2.8×10^1	7.0×10^2	1.5×10^3	1.4×10^3
12 h photoperiod						
pH 6	1.1×10^1	2.2×10^2	2.0×10^3	7.3×10^3	4.9×10^3	$1.7 \times l0^s$
pH 7	2.0×10^2	2.5×10^2	4.3×10^2	1.2×10^3	1.5×10^4	4.3×10^4
pH 8	1.9×10^1	1.7×10^2	1.6×10^3	1.4×10^3	1.2×10^4	5.6×10^4
24 h photoperiod						
pH 6	1.5×10^2	4.3×10^2	1.5×10^2	4.9×10^3	1.1×10^4	4.8×10^3
pH 7	2.3×10^3	1.8×10^2	2.3×10^3	3.2×10^4	1.1×10^4	8.5×10^3
pH 8	1.5×10^2	7.9×10^1	1.5×10^2	6.8×10^3	5.3×10^3	1.5×10^4

potato dextrose agar and malt extract agar media, where sporulation was best, 12 h photoperiod was found to be more suitable for abundant sporulation as compared to continuous dark or continuous light treatments (Tables 2 and 4). On Czapek dox agar medium, where sporulation was intermediate between the two extremes, both continuous darkness and 12 h photoperiod were found to be equally effective for better sporulation (Table 3). Most light sensitive fungi sporulate when exposed to continuous light, but some called diurnal sporulators, require a period of dark followed by a light period (Leach, 1967). Such fungi require light to initiate conidiophore formation and sporogenesis. However, completion of sporulation is inhibited by light. *Alternaria*, *Choanophora*, *Helminthosporium, Perenospora* and *Stemphylium* spp. are examples of diurnal sporulators (Dhingra and Sinclair, 1993).

Interaction of temperature, pH and photoperiod on growth and sporulation of *D. hawaiiensis*

The candidate fungus exhibited variable response in terms of growth and sporulation, to various employed ranges of temperature, pH and photoperiod regimes (Figures 1 to 5 and Tables 1 to 5). The interaction among these physical factors was found to be equally effective for growth and sporulation. On potato dextrose agar, 25 and 30°C at pH 6 under 12 h photoperiod was the best combination of environmental conditions for maximum sporulation. Difference in sporulation among the temperatures was significant at pH 6 and 8 under 12 h photoperiod and among different pH levels at 25 and 30°C under the same light conditions (Table 4). Maximum spore density was also recorded at pH 6 and 30°C under 12 h photoperiod (Table 4). The importance of interaction of temperature and light

Table 3. Effect of light, temperature and pH on sporulation in *D. hawaiinsis* on Czapek Dox agar medium.

Parameter	Number of spores (cm^{-2})			Total number of spores on the colony		
	20°C	25°C	30°C	20°C	25°C	30°C
Complete darkness						
pH 6	1.7×10^2	6.5×10^2	1.6×10^2	1.4×10^3	5.4×10^4	1.7×10^3
pH 7	3.1×10^1	1.8×10^2	1.5×10^1	2.1×10^3	2.2×10^3	5.3×10^2
pH 8	3.7×10^1	1.3×10^3	6.0×10^1	2.3×10^3	6.1×10^4	2.5×10^2
12 h photoperiod						
pH 6	6.7×10^1	4.9×10^4	2.5×10^1	4.0×10^3	4.1×10^4	2.4×10^3
pH 7	1.7×10^2	1.3×10^2	8.9×10^2	4.8×10^3	6.9×10^3	3.0×10^4
pH 8	2.5×10^1	5.0×10^1	1.6×10^2	8.0×10^3	3.1×10^3	1.4×10^4
24 h photoperiod						
pH 6	1.0×10^3	2.2×10^2	1.8×10^1	2.7×10^3	1.1×10^3	1.4×10^3
pH 7	4.0×10^2	1.3×10^3	1.2×10^1	1.2×10^3	5.5×10^4	5.3×10^4
pH 8	7.7×10^1	8.1×10^1	1.4×10^2	1.1×10^3	2.4×10^3	2.5×10^3

Table 4. Effect of light, temperature and pH on sporulation in *D. hawaiinsis* on potato dextrose agar medium.

Parameter	Number of spores (cm^{-2})			Total number of spores on the colony		
	20°C	25°C	30°C	20°C	25°C	30°C
Complete darkness						
pH 6	1.0×10^2	9.2×10^2	4.2×10^2	7.5×10^3	3.7×10^4	2.4×10^4
pH 7	3.2×10^2	2.0×10^3	3.6×10^2	3.7×10^4	5.7×10^4	2.0×10^4
pH 8	6.2×10^2	5.4×10^2	5.7×10^2	8.0×10^4	1.6×10^4	2.5×10^4
12 h photoperiod						
pH 6	3.7×10^2	2.1×10^3	2.1×10^3	4.1×10^4	2.4×10^5	2.3×10^5
pH 7	1.2×10^3	2.2×10^2	4.5×10^2	2.4×10^4	2.8×10^4	4.4×10^4
pH 8	3.4×10^2	3.8×10^1	7.9×10^2	3.9×10^4	4.1×10^4	7.5×10^4
24 h photoperiod						
pH 6	7.0×10^1	6.4×10^1	3.2×10^1	3.3×10^3	4.6×10^3	3.0×10^3
pH 7	3.1×10^2	1.9×10^2	0.0×10^0	1.3×10^5	1.2×10^5	1.1×10^5
pH 8	1.1×10^2	5.6×10^1	1.2×10^2	7.3×10^3	4.3×10^3	6.5×10^3

Table 5. Effect of light, temperature and pH on sporulation in *D. hawaiiensis* on Richard's agar medium.

Parameter	Number of spores (cm^{-2})			Total number of spores on the colony		
	20°C	25°C	30°C	20°C	25°C	30°C
Complete darkness						
pH 6	1.2×10^1	1.2×10^1	4×10^0	8.0×10^1	1.1×10^4	2.7×10^3
pH 7	3.5×10^1	1.5×10^1	2.9×10^1	8.0×10^1	6.7×10^3	6.7×10^3
pH 8	2.9×10^2	2.0×10^0	4.9×10^1	1.2×10^3	1.3×10^3	9.3×10^3
12 h photoperiod						
pH 6	4.4×10^1	2.1×10^1	6.0×10^0	1.1×10^3	1.5×10^3	8.0×10^2
pH 7	1.3×10^2	1.1×10^1	$0.0 \times I0^0$	1.7×10^3	5.3×10^2	0.0×10^0
pH 8	3.1×10^1	8.0×10^0	8.5×10^0	9.3×10^2	5.3×10^2	3.5×10^3
24 h photoperiod						
pH 6	3.6×10^2	1.5×10^1	1.1×10^1	4.3×10^3	4.7×10^2	1.1×10^3
pH 7	2.7×10^2	4.8×10^1	0.0×10^0	2.0×10^2	0.6×10^2	0.0×10^0
pH 8	2.3×10^1	4.0×10^1	9.7×10^1	0.2×10^2	9.5×10^2	5.3×10^2

for most of the diurnal sporulators is well documented. *Alternaria* and *Stemphylium* spp. form conidiophores under light at high temperature and conidia in the dark at low temperatures. Low temperatures may nullify the effect of light in some fungi (Aragaki, 1964).

The present study concludes that for the vegetative growth of *D. hawaiiensis,* all the five growth media were suitable. However, potato dextrose agar was found to be the best. On this growth medium, 25 and 30°C at pH 6 under 12 h photoperiod and on malt extract agar at 30°C under same conditions of light and pH were the best combinations to enhance the sporulation of the fungus. Findings of the present study will be helpful in mass culturing of *D. hawaiiensis* and used as mycoherbicide for the management of *M. minuta* under field conditions.

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