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EFFECT OF LIGHT ON GROWTH AND DEVELOPMENT OF PLASMODIA IN *DIDYMIUM NIGRIPES*

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The effect of light on growth and sporulation in *Didymium nigripes* was studied and it was found that light stimulated the plasmodia formation as well as sporulation in this species.

Keywords : Myxomycetes; Moist chamber technique; Plasmodia; Sporulation.

Introduction

Very few species of myxomycetes are known which have been cultivated in the laboratory on artificial media from spore to spore[1]. Most of the attempts were not successful as culture plates were often contaminated with other microbes like fungi, protozoa and nematodes. Although a chemically defined medium was given by Daniel and Rusch[2] for studies concerning nutritional requirements of myxomycete species yet in general there is still very little information is available in the area. In the present study, pure cultures of D. nig*ripes* were used as test material to see the effect of light on its growth and development.

Material and Methods

Plasmodium of *D. nigripes* (Link) Fries was obtained from decaying leaves and pieces of bark collected from Botanical Garden, Delhi University and old Delhi ridge, a natural forest stand adjoining the University, using both the moist Chamber Technique[3], as well as the agar Method.

The plasmodium was also grown on nutrient media. A preliminary examination for the selection of media which could support better plasmodial growth without contamination was made by using several media namely Corn Mela Agar, Knop's Agar and SM/5 Agar. Corn meal agar was found to be the best medium for growth and sporulation. Efforts were made to avoid any contamination by sealing the Petri plates so that no microbes from air could enter the culture plates. When the plasmodia first became apparent, a piece of agar with free vein end of plasmodium was cut (inoculum) and transferred to a fresh plate of agar. The process was repeated until the plasmodium was free from fungal and other contaminants like bacteria. Experiments were then carried out to study (i) effect of light interval/ duration, and (ii) effect of quality of light.

Effect of light interval/duration: Different sets of Petri plates were prepared to see the effect of light duration on growth of plasmodia and sporulation.

(1) Petri plates were kept for 3 hrs. in dark after inoculation and then shifted to continuous light.

(2) Petri plates kept for 6 hrs. in dark after inoculation and then shifted to continuous light.

(3) Petri plates kept for 24 hrs. in dark after inoculation and then transferred to continuous light.

(4) Petri plates kept for 24 hrs. in dark after inoculation, shifted to light for 3 hrs and then again shifted to continuous dark.

(5) Petri plates kept in continuous light after inoculation.

(6) Petri plates kept in continuous dark after inoculation.

Six replicates were taken for each treatment. Light source used was one 40 w fluorescent tube (500 lux) 30cm above the cultures. For dark treatment, Petri plates were wrapped in black paper and kept in dark.

Effect of quality of light: An experiment was conducted under similar conditions except for the quality of light used. Petri plates were kept under different light conditions. Cellophane papers of three different colours namely red, blue and green were used for this purpose. Light was obtained from one fluorescent tube filtered with four layers of these cellophane.

Assessment of Plasmodia growth : Since it is very difficult to lift the plasmodium and hence measure its size, the total protein content of plasmodium per petriplate was measured as an index of growth. Each value of protein content represents the average of four replicates estimated following the procedure of Lowry *et al.*[4] and expressed as µg/plate. Percent frequency of sporulation was calculated by taking the number of sporulated plasmodia to the number of total plasmodia considered X 100.

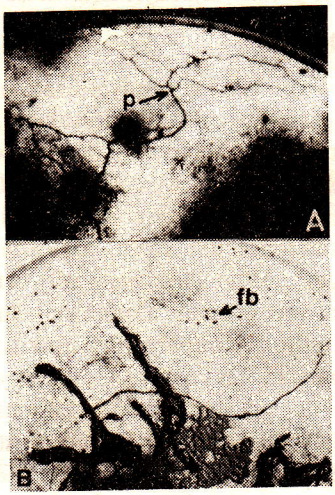

Fig.1 (A) : Petri plate showing plasmodium (p) of *Didymium nigripes* after 7 days of incubation.
(B) : Same Petri plate showing fruiting bodies (fb) after 9 days of incubation.

Table 1. Effect of Light/dark treatment on vegetative growth after 2 and 9 days of incubation and sporulation in *D.nigripes*.

Light/dark treatment	Plates showing plasmodia after		% frequency of Sporulation
	2 days of incubation	9 days of incubation	
3 hrs. dark -CL	2/6	4/6	33
6 hrs. dark -CL	5/6	6/6	33
24 hrs dark -CL	0/3	2/6	33
24 hrs.dark-3 hrs.light -CD	0/6	2/4	-
Continuous light	6/6	6/6	75
Continuous dark	0/6	4/6	-

-No sporulation; CL-Continuous light; CD-Continuous dark

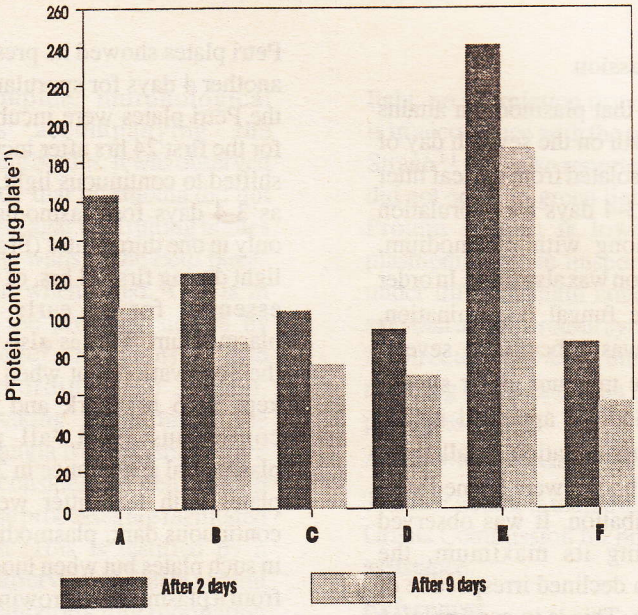

Fig. 2. Total protein content after 2 days and 9 days of incubation in *Didymium nigripes* cultured at different duration of light. A, 3-h dark-CL; B, 6-h dark-CL; C, 24-h dark CL; D, 24-h dark-3 h light-CD; E, CL; F, CD.

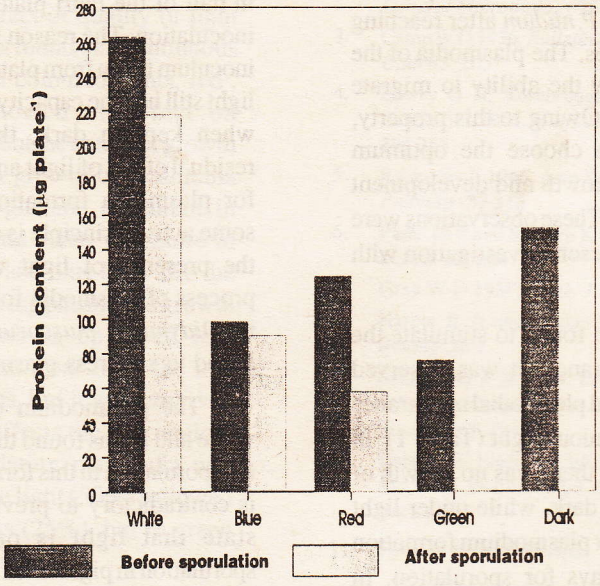

Fig. 3. Total protein contents before and after sporulation in *Didymium nigripis* cultured at different light conditions. There is no sporulation under green light and dark.

Results and Discussion

The study reveals that plasmodium attains its maximum growth on the seventh day of incubation, when isolated from the leaf litter and took another 3-4 days for sporulation (Fig.1 A&B). Along with plasmodium, fungal contamination was also there. In order to minimize these fungal contamination, the plasmodium was subcultured several times on the same medium under similar conditions. Plasmodium appeared in 2-3 days with less contamination in all these plates and fruiting bodies were formed after 8-10 days of incubation. It was observed that after reaching its maximum, the plasmodial growth declined irrespective of cultural conditions. This is in confirmation with Daniel and Rusch[2] and Rakoczy[5] who reported that growth in Physar*um polycephalum* and *P.nudum* after reaching an optimum declines. The plasmodia of the myxomycetes show the ability to migrate over a substratum. Owing to this property, a plasmodium can choose the optimum conditions for its growth and development in the environment. These observations were confirmed in the present investigation with *D.nigripes.*

Light has been found to stimulate the plasmodium appearance. It was observed that all plates showed plasmodial appearance when kept in continuous light (Table 1). In initial experiments, there was no growth of plasmodium in the dark, while under light it took 5 to 6 days for plasmodium formation and another 3-4 days for sporulation. In later studies under continuous light it took just one day for plasmodia formation and all Petri plates showed its presence and it took another 4 days for sporulation. If however, the Petri plates were incubated under dark for the first 24 hrs after incubation and then shifted to continuous light, it took as much as 3-4 days for plasmodia formation, but only in one third plates (Fig.2). It seems that light during first 24 hrs. of the incubation is essential for an early appearance of plasmodium. This is also confirmed from the observation that when plasmodia were kept in 6 hrs dark and then shifted to continuous light, all plates showed plasmodial appearance in 2 days. When the plates with leaf litter were incubated in continuous dark, plasmodia did not appear in such plates but when inoculum was taken from a plasmodium growing in the light and subcultured in separate Petri plates and incubated in the dark, plasmodia appeared in half of the Petri plates within 2 days of inoculation. The reason for this may be that inoculum taken from plate kept in continuous light still had the capacity to form plasmodia, when kept in dark, thus indicating the residual effect of light and necessity of light for plasmodia formation. It appears that some active principle is synthesised only in the presence of light which initiates the process of plasmodia formation. However, in *Didymium muscorum*, light has been found to suppress plasmodial growth[6].

The plasmodium of D. *nigripes* is white and it was found that light is essential for sporulation in this form. This observation is contradictory to previous studies which state that light is only essential for sporulation in pigmented myxomycetes[7] and it is not required in non pigmented plasmodia.The reason may be that light is

required to complete morphological transformations accompanying the formation of fruiting bodies. It was observed in the present study that plasmodia did not sporulate on the wet agar but fruiting bodies were formed on the walls of the Petri plates or on the medium when dried. This is in accordance with the previous studies by Harper and Dodge[8], Skupienski[9] and Daniel[10]. According to them, transformations taking place during the formation of sporangia from plasmodia are accompanied by considerable dehydration of cytoplasm. These processes are facilitated by a dry medium. This is testified by a afore-mentioned observations that in natural conditions the plasmodia before sporulation migrate to dry substrate and in laboratory culture to the walls of the Petri plates or on dry medium. The effect of quality of light was studied and it was found that continuous exposure to different coloured lights, red , blue and green showed no effect on plasmodia formation but the extent of growth varied i.e. maximum growth of plasmodia occurred in white light and minimum in green light. Also, time taken was more in the latter case. In red and blue light also the number of plates showing plasmodial growth was less and plasmodia appeared less extensive. This is also clear from the low protien concentrations, but it was observed that quality of light affects the sporulation. Percentage of sporulating plasmodia was maximum in the white light (50%) followed by blue and red lights (25%), but in green light, no sporulation occurred (Fig.3). This is in accordance with the previous studies of Straub[11] who also reported that sporulation did not occur in green light in D. nigripes . Protein content is lower in sporulated plasmodia than in unsporulated plasmodia under different light conditions. It can be said that all the protein synthesis at this time is directed towards metabolic changes resulting in the initiation of morphogenetic processes.

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