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ECOLOGICAL AND PHYSIOLOGICAL STUDIES ON EGYPTIAN CRYPTOGAMS : I-EFFECT OF SPECTRAL QUALITY OF LIGHT ON SPORE GERMINATION AND PROTONEMA DEVELOPMENT IN FUNARIA HYGROMETRICA HEDW.

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ABSTRACT

The effects of light of different wavelengths on spore germination percentage and protonema development have been studied in Funaria hygrometrica Hedw. The regulatory role of light of different spectral composition in gametophore formation and controlling spore germination is discussed.

The main points concluded from this investigation are :

1 - Spore germination percentage appears to be environmentaly related, here, to light quality and edaphic factors.

2 - Spore germination percentage increased with the increase in wavelength (i.e. from blue to red).

3 - Protonemata and gametophyte formation were retarded in blue light and inhibited in dark and in FR.

4 - Spore germination obtained on unsterilized medium showed much better growth.

INTRODUCTION

The promoting effect of light on moss spore germination was first discovered by Borodin (c.f. Bauer & Mohr, 1959). In an attempt to identify the photoreceptors concerned with the induction of germination by light, a number of workers, pointed out that a phytochrome system is involved in the control of germination in *Funaria hygormetrica* and *Physcomitrella patens* since the

germination is enhanced by red light and the effect is reversed by immediate exposure to far - red light (Bauer & Mohr, 1959). Other workers studied the effect of light quality on bud induction in archegoniates (Mitra *et. al.*, 1959; Mohr, 1963; Rashid, 1970; Cove et. al., 1978 and Dietert, 1980). They found that red light was more effective than either blue or green light in inducing buds in *Funaria hygrometrica*, *Pohlia nutans* (Hedw) lindb. and *Anoectangium thomsonii* Mitt.

Although the course of germination has been studied in many spores in the world (Valanne 1966, 1971; Karunen 1972; Paolillo & Kass, 1973; Olesen & Mogensen, 1978), little is known about the needs of the germinated spore for maintaining life while awaiting suitable conditions for protonema growth. It is assumed,however (Valanne, 1971), that the majority of spores within a relatively short period will either initiate protonema formation or die. Since a combination of such common factors as water and light can activate germination and protonema growth in many taxa of bryophytes, most spores probably germinate shortly after dispersal has occurred.

The factors affecting the length of time necessary for the germination of moss spores of several species including *F. hygrometrica* are the condition of the spores, the culture medium used, illumination and difference in temperature (Lesage, 1918). Marchal & Marchal (1906) concluded that the spores of *F. hygrometrica* germinate in distilled water, tap water, and Marchal's solution, or on Sphagnum soaked with any one of the three liquids. Germination of spores starts firstly by swelling due to the absorption of water, secondly the increase in amount of chlorophyll, and thirdly the change in the shape of the spores. This change in shape

is represented in the pushing out of a papillate protrusion from one side or more of the spore. The exospore ruptures at the apex of such protrusion which develops into a protonema.

As far as we know, the volume of literature concerned with the effect of light on spore germination and protonema development in cryptogams (especially mosses), is small and that concerned with the effect of the spectral quality of light on spore germination of this group of plants is even more scarce.

It is worthy to mention that except for the work done by El-Saadawi & Badawi (1977) and Abou El-Kheir et. al., (1986, 1988) on the algal-moss association and that by Ghanem (1983, 1986) on the ecology and eco-physiology of some Egyptian mosses including F. hygrometrica, no other ecological or eco-physiological studies on bryophytes have so far been done in Egypt.

Funaria has claim to be considered a "Famous plant" because it has served for very many years as the type for the study of the structure and life cycle of a moss (Watson, 1978). Moreover, in recent years, it has been utilized in an increasing number of fields of research, not all of them are strictly bryological. The species F. hygrometrica has been chosen, here, as a material for the first part of a series of researches, to study the effect caused by spectral light, as one of the environmental factors, on the germination of spores and the development of protonemata.

MATERIAL AND METHODS

A. Material :

One sample of fruiting Funaria hygrometrica was collected from the surface

of a wet red-brick wall of an old house in El-Khanka district, in Qaliobyia governorate on 15.7. 1990. Capsules contained ripe spores that grminate, in a few days, when wetted with water.

B. Methods

B. 1. Culture media :

Two culture media were used in the germination of spores, viz.

B. 1.1. Marchal's solution : This medium is composed of :

Distilled water.....	1000 c.c.
Ammonium nitrate.....	1.00 gm
Potassium sulphate.....	0.50 gm
Magnesium sulphate.....	0.50 gm
Calcium sulphate.....	0.50 gm
Ammonium phosphate.....	0.50 gm
Iron sulphate.....	0.01 gm
Potassium hydroxide.....	(10% solution-few drops)

The culture fluids were placed in Erlenmeyer flasks and sterilized in the autoclave for 30 - 60 minutes at 1.5 pounds pressure. This medium also was used without sterilization for the germination of spores.

B. 1.2. Soil extract medium :

This medium was first used by Ghanem (1983). It consists of soil extract 0.1% (w/v. in distillded water), 100 ml, sodium nitrate, 0.2 gm and agar, 15 gm.

Soil extract was prepared by suspending 1 gm fresh garden soil in 1000 ml distilled water. The soil suspension was digested (autoclaved at 1.5 atm. for 15 minutes) for extracting the nutrients present in the garden soil. The soil extract was then filtered using Buchner funnel and Whatmann filter paper No. 1. To the filtrate 0.2 gm sodium nitrate and 15 gm agar were added. The medium was autoclaved at 1.5 atm for 15 minutes. Sterile petri-dishes containing such medium were used for culturing spores of F. *hygrometrica*.

B. 2. Preparation of spore suspension :

To avoid contamination of the prepared cultures with air borne microorganisms, spores ..... etc., the capsules were surface sterilized using 5% mercuric chloride solution (15 gm mercuric chloride mixed with 20 ml HCl and 100 ml distilled water) then washed with sterile distilled water several times. It is convenient to separate the sporophyte from the gametophyte at the base of the seta, leaving the stalk to be used as a handle for manipulating the sporophyte during washing of capsule and sowing of spores. After surface sterilization, the undehisced capsules were reuptured by means of needles sterilized in Bunsen flame burner, the spores were then released and scattered in a certain volume of sterilized distilled water in a measuring flask under aseptic conditions (see Schelpe, 1953, Ward, 1960; Basile, 1967).

B. 3. Treatment of the inoculated media :

Experiments were designed for the study of the effect of spectral quality of light (Red "RL", Yellow "YL" Green "GL", Blue green "BGL", Blue "BL" and Far-red "FR" radiations) that transmitted to the surface of each inoculated dish.

These radiations were given by covering the petri-dishes containing spore cultures (media) with a double layer of blue, blue-green, green, yellow and red cellophane papers, and exposing them to 40 W fluorescent tubes fixed at a distance of 40 cm from the culture media (as in Miller and Miller, 1964). The FR radiation was obtained from 100 W tungsten lamp where the dishes were covered by two layers each of red and blue cellophane paper. The temperature of the cultures was maintained at 28°C in an incubator.

The inoculated dishes were divided into three groups, each group consists of 16 petri-dishes representing 8 treatments : White (control), RL, YL, GL, BGL, BL, FR radiations and dark.

a - The first group includes spores grown on filter paper wetted with tap water.

b - The second grown on sterilized Marchal's fluid.

c - The third grown on unsterilized Marchal's fluid.

After three days of inoculation, the contents of each petri - dish were transferred to another dish containing solidified soil extract medium. A pair of dishes of each group was wrapped with aluminum foil to exclude light (dark treatment).

All the dishes were unwrpped every two days under sterilized conditions in sterilized glass room to examine the spores and permit gaseous exchange.

B. 4. Count of spores :

The counting of the whole number of spores in spore suspension was carried

out by means of haemacytometer. Petri - dishes were found to be more convenient than Erlenmeyer flasks as containers for the culture media because of the ease of count of germinated spores and gametophores.

The observation and count of germinated spores were carried out every two days and the final percentage of spore germination was calculated on the basis of the final number of spores germinating on the same medium when exposed to white light in the same conditions of light intensity and temperature.

The appearance of the first oblique septum of each protonema was taken as criterion of germination. To facilitate the counting of germinated spores a) The back of each inoculated dish was divided into four equal sections b) Small volume of each culture medium was poured - to give thin layer of media - in each petri - dish, (c) A certain volume of spore suspension (± contains the same number of spores) was distributed under aseptic conditions on the surface of each culture medium by means of sterilized pipette.

OBSERVATIONS AND RESULTS

Germination of spores of *F. hygrometrica* is indicated, here, by spore wall protrusion caused by the aperture apparatus (see photo. 1).

Spores on the three used media showed the highest percentages of germination with RL. Percentage of germination varied from 6 - 60%, 2 - 75% and 0.0 % - 57 % on sterilized & unsterilized Marchal's fluids and tap water respectively (see Fig. 1). It was observed that the germination percentage in each medium increased progressively with increase of wavelength (from blue light "BL" to red one "RL" (see photos 1 & 4). No germination was observed in both dark and FR radiation treatments even after 45 days (see photo 5).

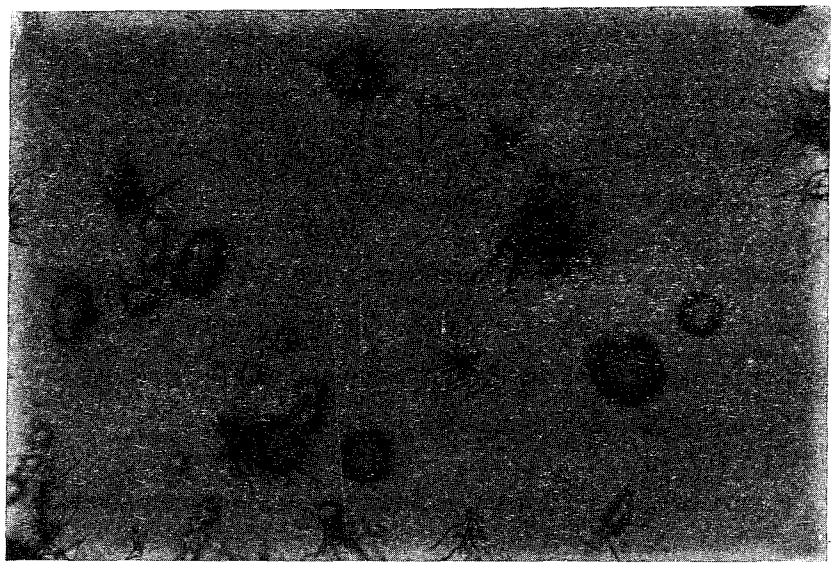

Photo. (1) : Showing spore germination of F. hygrometrica on unsterilized Marchal's medium, associated with fungi, under the effect of red light.

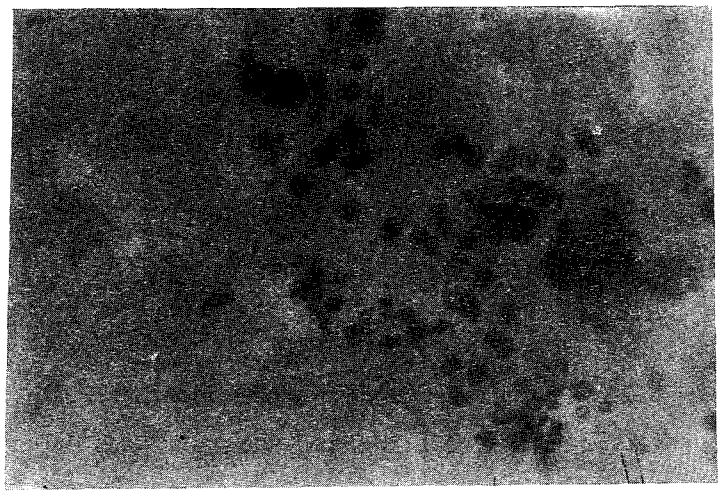

Photo. (2) : Showing the beginning of spore germination on filter paper wetted with tap water

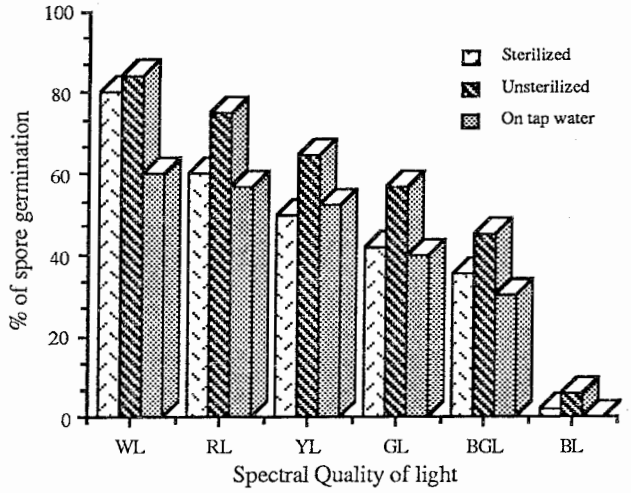

Fig. (1) : Percentages of spore germination in B F. hygrometrica as affected by spectral light quality (white "WL" red "RL", yellow "YL" green "GL", blue-green "B.GL" and blue "BL".) on the three used media.

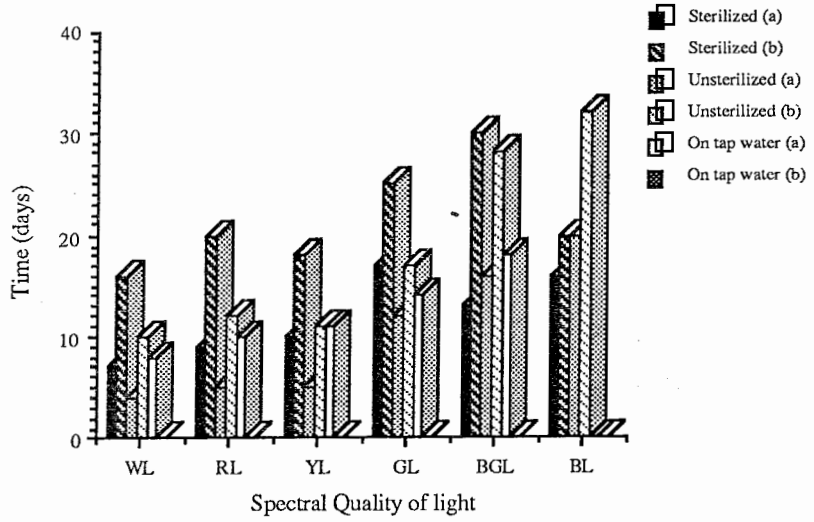

Fig. (2) : Time passed before the formation of the first septum on the protonema of F. Hygrometrice developed on the used media, and before the formation of gametophore (extensions).

Table 1: Percentage of spore germination in *F. hygrometrcia* (as affected by spectral light quality) on sterilized & un sterilized Marchal's fluid and tap water.

light Qty % germ.	WL	RL	YL	GL	BGL	BL
on Marchal's fluid - Sterilized	80.0	60.0	50.0	42.0	35.0	02.0
Unsterilized	84.0	75.0	65.0	57.0	45.0	06.0
on tap water	60.0	57.0	52.0	40.0	30.0	00.0

Table 2: The time passed from the inoculation of spores till the appearance of the first septum of protonemata and gametophores formation on the three used media (Experiments lasted for 40 days..

Time passed (days)	light Qty	WL (control)	RL	YL	GL	BGL	BL
Sterilized	Protonema	7	9	10	17	13	16
	gametophores	16	20	18	25	30	20
Unsterilized	Protonema	4	5	5	12	16	20
	gametophores	10	12	11	17	28	32
Tap water	Protonema	8	10	11	14	18	--
	gametophores	--	--	--	--	--	--

Our experiments (see table 1, Fig. 1) showed that the highest percentage for each spectral quality of light was recorded in case of spores germinated on unsterilized Marchal's fluid. Whereas the lowest percentage for each light quality (except YL.) occurred in case of those germinated on tap water (see photo 2, Fig. 1).

Under the same conditions of light intensity and temperature, spores exposed to white light (control) on the three used media showed germination percentages ranging from 60% (on tap water) to 84% (on unsterilized medium).

The length of time required for the appearance of the first septum as affected by visible radiation was variable . It varies from 5 - 20 days on the three media under the effect of light spectrum (see table 2 & Fig. 2).

The length of time from the first indication (protrusion) of germination till the formation of the first septum of the protonema (as affected by visble radiation), was almost the same, being 4 - 6 days, on the three used media. Whereas the time spent for those exposed to white light (control) varied from 4 - 8 days on the three media.

Regarding the time passed from the formation of the first septum of protonema - on sterilized and unsterilized media - till the formation of final number of gametophores (till 40 days after soaking of the spores), (photo 3 a & b) it was found that this time ranged between 5 & 8 for RL, YL and GL, while it ranged between 4 & 17 for BGL and BL.

Results presented in table 3 show that the final number of gametophores arising from buds on protonemata (till 40 days after soaking of the spores) ranged between 3 (with BL) and 31 gametophores (with YL) in case of sterilized

medium, and varied from 8 (BL) to 36 gametophores (with RL).

It was found that the final number of gametophores - under the effect of white light (control) - ranged from 40 (on sterilized meduim) to 50 gametophores (on the unsterilized one).

It was observed that none of the gametophores had arisen in case of tap water - under the effect of light quality - till 40 days after soaking.

Table 3: Showing the final numbers of gametophores formed on sterilized & unsterilized Marchal's fluid - as affected by spectral quality of light.

light Qty Marchal's fluid	WL (contrl)	RL	YL	GL	BGL	BL
Sterilized	40	30	31	22	20	3
Un-sterilized	50	36	33	18	15	8

DISCUSSION AND CONCLUSION

The observations and results embodied in the previous part highlight the major importance of spectral quality of light in controlling spore germination and protonema development in the common moss, *F. hygrometrica*

The highest percentages of germination and the most luxriant growth of protnemata resulted when the spores were sown on Marchal's solution are in harmony with that mentioned by Lesage (1918).

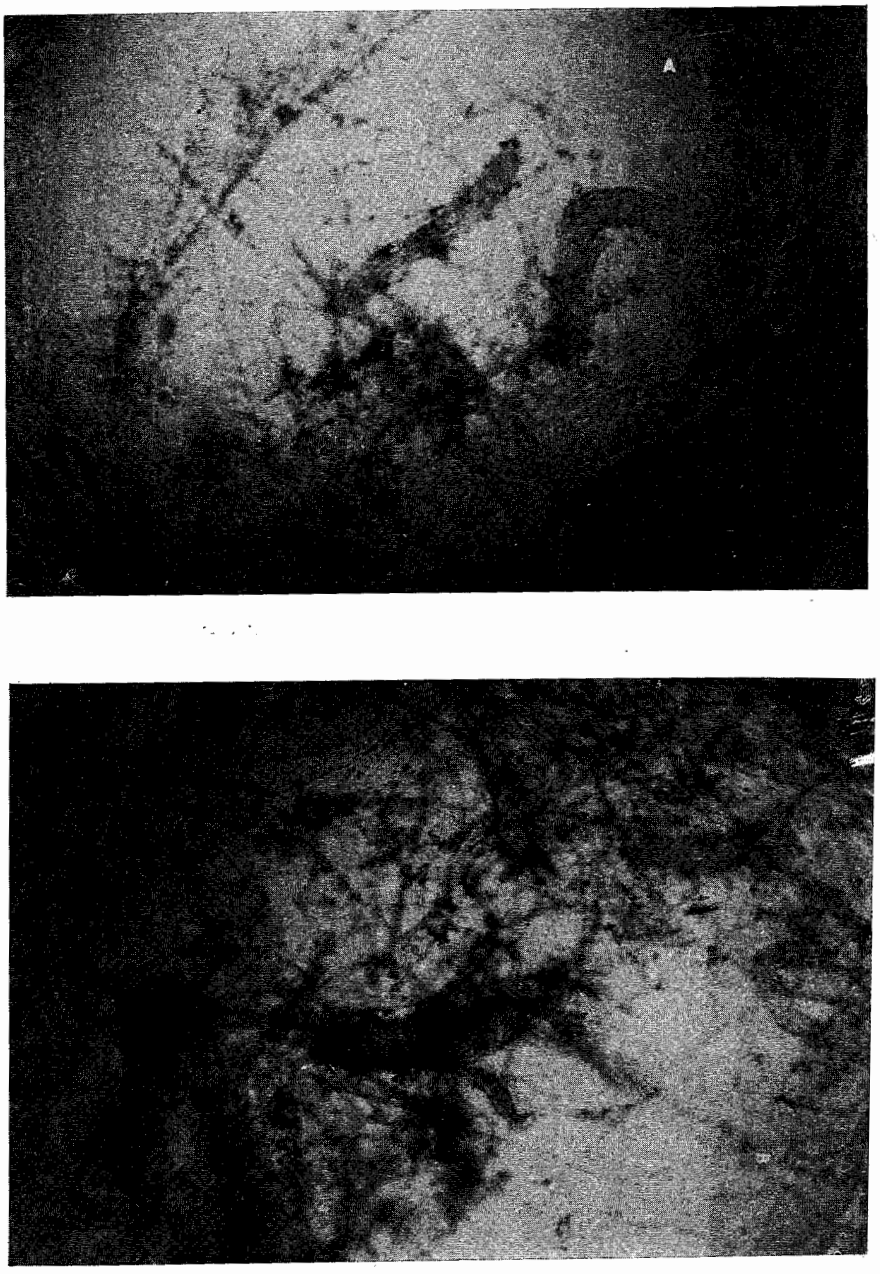

Photo. (3) : Showing gametophores developed from protonema on unsterilized medium under the effect of yellow light (A) and red light (B).

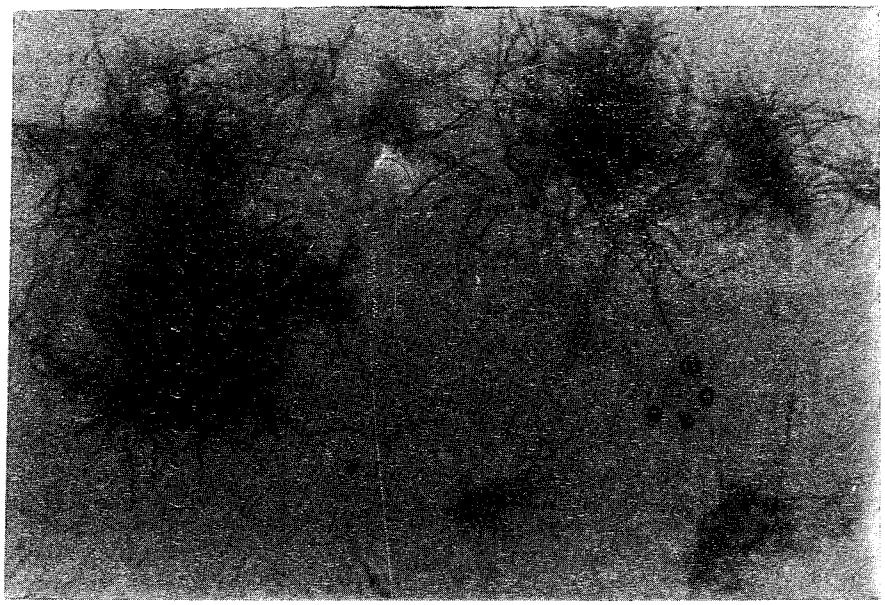

Photo. (4) : Showing the effect of blue light on spore germination on unsterilized medium after two weeks of inoculation.

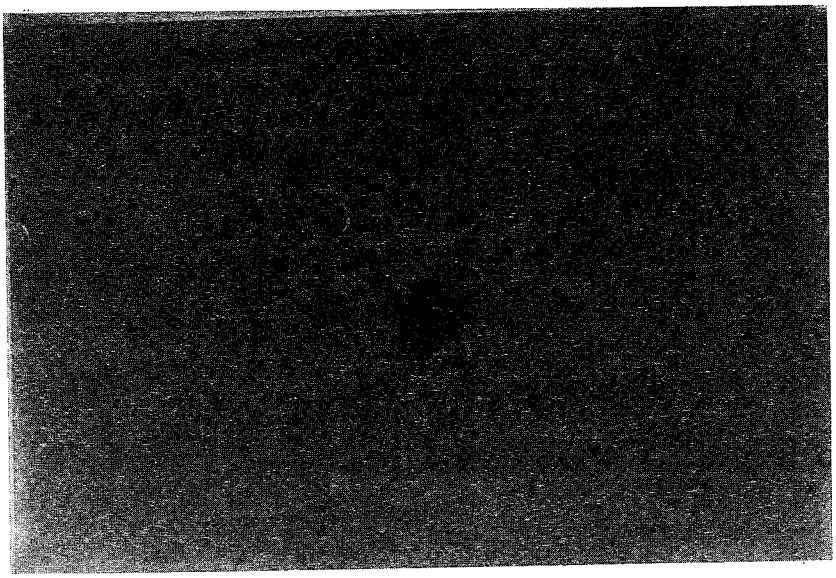

Photo. (5) : Showing the aggregated ungerminated spores left in dark.

The length of time required for germination of spores was somewhat variable. It was noticed that when spores were sown on unsterilized Marchal's fluid, germination took place - under constant temperature (28°C) as affected by white light (control) - within four days, while those sown on sterilized one showed no evidence of germination in less than about seven days. This observation can be explained in the light of the studies done by some workers (Dyer & Duckett 1984 and Lal, 1984). They mentioned that almost all fungi and other microorganisms occurring as contaminants in Bryophyte cultures appeared to have more or less promotory effects on the germination of spores and growth of protonemata. Similarly, Vaarama and Taren (1959) had observed that the normal germination of spores of the moss; *Tetraphis pellucida* Hedw occurred only when inoculated simultaneously with spores of Aspergillus, Fusarium and Mucor. Also, in Pogonatum. inoculation of spore cultures with Penicillium resulted in much better growth.

A comparison of the effect of light quality (of different wavelengths) on spore germination in *F. hygrometrica* indicates that the percentages of spore germination increased with increase in wavelength from blue (BL) light to red (RL) one, with retardation in protonema growth in blue (BL) light and inhibition of germination with FR radiation. This finding is in accordance with that declared by El-Tantawi (1989). The inhibition caused by FR shows the destructive action of short wavelength radiation (see Popay & Robert, 1970; Cruden, 1974 and Rao, 1988). This could be attributed to photocyberentic processes receptor substances which are chemically altered following uptake of radiation quanta. This alteration affects the control of metabolism, growth and development (Schulze and Klein, 1963; and Larcher, 1983).

The retardation of spore germination and bud formation with bule light can be explained by its inability to generate sufficient Pfr (phytochrome, maximum absorbance at 730 nm) for bud induction (Hilman, 1967). The same author added that blue light acts by shifting the ratio of Pr (absorbing maximally at 660 nm): Pfr.

Chhabra and Malik (1978) in work on *Arachis hypogaea.* concluded that blue light is involved in the destruction of endogenous IAA thus causing a reduction of pollen tube length. This possibility is further supported by the fact that the activity of IAA - oxidase enzyme enormously increased in the presence of BL.

Generally, blue light has been found to have variable effects on spore germination (Miller, 1968), but the exact mechanism of action of blue light is not clear.

Finally, the body of information concerning the effect of spectral quality of light on spore germination and gameophyte growth in Bryophytes, Pteridophytes and Gymnosperms, is rare or scanty. So, both field work and laboratory research are seriously needed.

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دراسات بيئية وفسيولوجية على نباتات لابذرية مصرية

١ - تأثير الضوء عند أطوال موجية مختلفة على أنبات الجراثيم ومرحلة البروتونيما فى فيوناريا هيجروميتريكا هيدو

الملخص عربى

تم دراسة تأثير الأطوال الموجية للضوء على إنبات الجراثيم ومرحلة البروتونيما فى حزاز فيوناريا هيجروميتريكا هيدو. ولقد ناقش البحث الدور المنظم للضوء ذو التكوينات المختلفة على نمو الجراثيم .

ولقد خلص الباحثون الى النقاط التالية :

١ - إرتباط النسبة المئوية لانبات الجراثيم بالعوامل البيئية مثل نوعية الضوء .

٢ - زيادة نسبة الجراثيم المنبته بزيادة طول الموجه .

٣ - إرتباط البروتونيما بتكوين الطور المشيحى فى الضوء الأزرق بينما تتوقف فى الاحمر .

٤ - حدوث نمو جيد فى حالة إنبات الجراثيم على أوساط غذائية غير معقمه .