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A STUDY OF THE BIOLOGY AND PHYSIOLOGY OF *HELMINTHOSPORIUM BICOLOR* ISOLATED FROM *STENOTAPHRUM SECUNDATUM*

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ABSTRACT

The study of the biology and physiology of *Helminthosporium bicolor* isolated from leaf lesions of its host *Stenotaphrum secundatum* showed good mycelium knowledge on complex media as well as on synthetic media rich in minerals and vitamins. This fungus was able to develop, specifically, on complex environments and less on synthetic media. Rice flour media, PDA, and Richard's Czapeck proved conducive to the growth and sporulation of this pathogenic agent. The light regime is favorable to mycelium growth and sporulation. Optimal temperatures for growth and sporulation of *H. bicolor* are of 20 and 28°C for the pH which ranges between 6 and 8.

KEYWORDS: Biology, Physiology, *Helminthosporium Bicolor, Stenotaphrum Secundatum,* Pathogen, Mycelium Growth

INTRODUCTION

Helminthosporium disease is regarded as an important cryptogrammic disease. The form of *Helminthosporium* includes several species of which one the most popular includes: *H. sativum, H. maydis, H. zeicola, H. spiciferum*… These species can, according to some scholars, be more frightening than *H. oryzae.* Rice Helminthosporium disease caused by H. oryzae, was first referred to in 1900. This disease is regarded as the second dangerous rice disease from the point of view of its importance after the pyriculariosis generated by *Pyricularia oryzae* (Ou, 1985). The output effects caused by this disease can reach 75% (Kohls and Percich, 1983; Kohls et al., 1987) and even 100% in the event of severe epidemics (Bean and Schwartz, 1961; Kernkamp et al.; 1976).

Fungi isolation from the infected rice plants, extracted from the Moroccan rice plantations, showed the existence of various medical problems. Thus, the helminthosporium and the pyriculariosis seem to be a fungal disease predominant on the stems, leaves and seeds (Benkirane et. al, 1994; Benkirane, 1995). In general, important rice foliar diseases are classified in the large category of helminthosporium which includes not only the diseases caused by *Helminthosporium* but also by other organisms such as: *Curvularia, Trichonis, Brachysporium* etc. These fungi have approximately the same biology and cause similar deteriorations and infections not only on the foliar organs, but also, on the floral parts (glumes and glumellas). This causes seeds contamination (Angladette, 1966).

In Gharb and Larache area, we think that the population of the various foliar parasites is much diversified. The existence of numerous weeds and other plant species in the Moroccan rice plantations can be the cause, among many others, of the presence of various species of parasites, including *Helminthosporium bicolor. Stenotaphrum secundatum*

happens to be the host of most rice diseases. Benkirane's (2001) study on compared pathogenesis and the isolate cross inoculations of *Pyricularia grisea (Magnaporth grisea)* reveals that certain varieties of rice are sensitive to weed isolates and that certain species of the herbaceous plants are sensitive to rice isolates.

Helminthosporium bicolor (Mitra 1931), or *Dreschlera bicolor* was discovered in Poona (India) at the same period with the discovery of the decay of corn plants. Since then, this fungus has not been the subject of thorough investigation. The *H. bicolor* spore is of more or less small size, pertaining to the family of bicolor Helminthosporiums, having size similar conidia to those of *Helminthosporium carbonum, Helminthosporiums setariae Saw* and *Helminthosporium cynodontis Marig*. The conidia are straight or slightly curved, with brown aspect or ashes with two sub-hyaline final cells. This bicolor aspect is a distinction criterion of this species and makes the latter a particular species (Mitra, 1931). In this paper, we are concerned with the study of this fungus, which has been rarely inquired into so far.

The development of the various species of *Helminthosporium*, isolated from the affected rice plants, is evaluated through the growth, the diametrical and spore production. The development capability of these fungi on various areas informs about their aptitude to colonize various ecological niches. In addition, their capability to sporulate makes it possible to determine their distinction in nature (Hsiech et. al, 1979; De Cal et al., 1993). The physiological study makes possible, the distinction between the isolates of the same fungal species. This has been the subject of various studies on different fungi (Engelhard et al., 1972; Okeke et al., 1992a; Benkirane, 1995)

After having isolated from *Stenotaphrum secundatum H. Bicolor*, we set ourselves the goal of studying, biology (the behavioral study of the *Helminthosporium bicolor* isolates on various complex and synthetic culture media), physiology (the study of two physical factors effect, pH and the temperature on the of *Drechslera bicolor* growth and the sporulation) and the pathogenic capability of this fungi on rice and *Stenotaphrum secundatum.*

MATERIAL AND METHODS

Helminthosporium Bicolor Isolate Used

Two isolates of *Drechslera bicolor* are used in this study: Dbc or Dbc1 and Dbe or Dbc2. These various isolates are obtained from foliar lesion of *Stenotaphrum secondatum* plants harvested in the neighborhoods of Kenitra. *Stenotaphrum secondatum* leaves are cut out in small fragments of approximately 2cm² on the level of the lesions caused by different pathogens. These fragments are soaked thereafter in bleach for 5 minutes and are rinsed five times in the sterile distilled water. They are then placed in Petri dishes containing each three paper discs humidified with sterile distilled water. After seven days of incubation, monosporale isolations are carried out under microscope with a rolled glass wire capillary, previously sterilized with fire and cooled. These spores are automatically deposited in Petri dishes containing of gelosee water (2%), then transferred in an environment containing flour freshly prepared rice flower. After 72 hours at least, a clone is chosen and multiplied by transplanting on the medium, and the identification is carried.

Influence of the Culture Medium

Though the study of the growth of the two tested H. bicolor isolates, two types of culture media are used: -Complex medium: it includes Potato Dextrose Agar (PDA), rice flour medium and the extract of Malt and oat flour. Synthetic medium: it includes the Czapeck medium, Richard and Richard's. For each medium and isolate, seven Petri dishes are sown by placing in their center a 5 mm diameter explants taken from the margin of a 7 days old culture. These Petri dishes are then incubated at a temperature of 25°C for 20 days in the light, in the darkness and alternatively 12 hours in the light

and 12 hours in the darkness.

pH Influence and Temperature

The medium used for the study pH effect and temperature on the mycelium growth and the sporulation of the tested isolates is the medium containing rice flour. For each pH (4, 5,6,7,8 and 9), each temperature (10°c, 15°c, 20°c, 28°c, 35°c, and 42°c) and each isolate, 12 of Petri dishes are sown the same way as previously.

RESULTS ANALYSIS

The diametrical growth is estimated by the measurement of the cultures according to two perpendicular diameters after 7 days of incubation with the temperature of 28°c for the various culture media, and 6 days of incubation for the temperatures and tested pH. The number of spores is estimated after 20 days of incubation. Four discs of 5mm diameters, taken from the edge of the cultures are deposited in a test tube containing 1ml sterile distilled water. The tubes are agitated with the vortex for 40 seconds so as to remove the spores. The evaluation of the total number of conidia released by the 4 discs is carried out through the cell of Malassez at a rate of ten counting per suspension of three boxes for each isolate. The median number of the counted spores is then expressed per unit of the surface of the thallus considered (median number of spores /mm ^{2}).

STATISTICAL ANALYSIS

The data processing of the diametrical growth and the sporulation of the isolates of H. bicolor on various, culture media, pH and T° C is carried out by a statistical software: Statistica and has to do with the analysis of the variance followed by the test of Student - Newman - Keuls (SNK°) to the threshold of 5% when the result of the analysis of the variance records at least a significant difference with the threshold of 5%.

RESULTS

Mycelium Growth and Sporulation of the Isolates of H Bicolor (Dechslera bicolor) on the Complex Media

Mycelium Growth

The results, drawn from the statistical analyses carried out on different data of the diametrical growth (Table 1 to 6) showed significant differences with the threshold of 5%, between the two H. bicolor isolates tested on the various media. In addition, the isolate effect of culture and isolate-medium is highly significant.

On the rice flour medium, under continuous light, the Dbc1 and Dbc2 isolates do not significantly differ between them. The two isolates have the same the diametrical growth: 85mm.

Table 1: Mycelial Growth (mm) of the 2 isolates of H. Bicolor in the Complex Mediums in Continuous Light

Isolate		
Culture Media	**Dbc1**	**Dbc2**
E. Malt	70a	61a
Oats	73a	66a
F. Rice	90a	90a
PDA	83a	82a

For the same medium, the values of the same line affected of the same letter do not significantly differ from the threshold of 5%

A significant difference is only at the level of the oats extracted medium for the two isolates: 70 mm for Dbc1 isolate and 61 mm for Dbc2 isolate. On the whole media, the diametrical growth varies between 61 mm and 90 mm. For the Dbc1 and Dbc2 isolates, the diametrical growth varies respectively from 70 mm to 90 mm and 61 mm to 90 mm on all the selected media. Nevertheless, significant differences have been observed for the same isolate within the various culture media. For the same Dbc1 isolate, the diametrical growth on the rice flour medium and PDA (90 mm and 83 mm respectively) significantly differ from that from malt extracted medium and oat flour (70 mm and 73 mm respectively). In the same way, for Dbc2 isolate, the diametrical growth relevant to rice flour and media (90 mm and 82 mm respectively) significantly differ from those extracted the malt and rice flour media from malt and rice flour (61 mm and 66 mm respectively). The maximum growth is observed on the level of the rice flour medium, and this, for the two isolates Dbc1 and Dbc2. The medium extracted from malt has revealed the lowest maximum growth for the two isolates. Within the darkness (Table IV.2), on the entire media, the diametrical growth varies between 70 and 80 mm for the Dbc1 isolate and 68 to 85 mm for the Dbc2 isolate. The PDA medium has shown a maximum mycelia growth for the two isolates (80 mm and 85 mm for Dbc1 and Dbc2 respectively). On rice flour, the diametrical growth of the Dbc1 isolate (70 mm) differs significantly from that of the Dbc2 isolate (82 mm). The diametrical growth of the Dbc1 isolate on the culture medium PDA (80 mm) differs significantly from that observed for this same isolate on the other media (70, 72,70 mm). As for the Dbc2 isolate, the diametrical growth on the mediums Extracts media(70 mm) and oat flour (71 mm) differs significantly from that observed on the other culture media (rice flour: 82 mm and PDA: 85 mm).

Table 2: Mycelium Growth (mm) of the 2 Isolates of H. Bicolor in the Complex Mediums in the Darkness

	Isolate	
Culture Medium	**Dbc1**	**Dbc2**
E. malt	70a	68a
Oats	72a	71a
Rice Flour	70b	82a
PDA	80a	85a

For the same medium, the values of the same line affected of the same letter do not significantly differ with the threshold from 5%

Alternatively (light-darkness), the rice flour medium is the most conducive to the mycelium growth of the two isolates (90 mm), its action on the mycelia growth significantly differs from that of the other culture media, on Dbc2 and Dbc1. The malt extracted medium seems the least conducive to the mycelia growth of the two isolates considered. As for PDA medium, it differs significantly from the malt extracted and oat flour media for the Dbc1 isolate and differs significantly only from the malt extracted medium with regard to the Dbc2 isolate. The study of the behavior of *H. bicolor* isolates enables us to say that the Dbc1 isolate experiences a considerable mycelium development on all the media compared to the Dbc2 isolate, the rice flour medium and PDA are conducive to the mycelium growth of the two isolates Dbc1 and Dbc2.

Table 3: Mycelium Growth (mm) of the 2 Isolates of H. Bicolor in the Complex Media in Alternation Light-Darkness

	Isolate	
Culture Medium	**Dbc1**	**Dbc2**
Malt extract	62.5a	59a
Oat	65b	68.7a
Rice Flour	90a	90a
PDA	70a	68.5a

For the same medium, the values of the same line affected of the same letter do not significantly differ with the threshold of 5%

The mycelium growth under continuous light is more considerable than the one under the darkness and the one alternatively under light and darkness. The minimum of growth is obtained on malt extracted medium and this, for the two studied isolates. The mycelium growth on the synthetic media is variously appreciated. Under continuous light, the Dbc1 isolate grows better than the Dbc2 isolate, and this, on the whole studied media. Its mycelium growth varies between 60 mm and 80 mm with a maximum growth on the Czapeck medium. The medium of growth is obtained on Richard medium for the isolate Dbc2 (59.5 mm). The Czapeck medium shows a significant difference when compared with the other media for the two isolates. The latter are significantly different with regard to this medium. For the two other media, we notice no significant difference between isolates.

Table 4: Mycelium GROWTH of the 2 Isolates of H. Bicolor in the Synthetic Media in Continuous Light

	Culture Media		
Isolats	Czapeck	Richard	Richard's
Dbc1	80a	60c	68b
Dbc2	72.5a	59.5c	66.4b

For the same medium, the values of the same line affected of the same letter do not significantly differ with with the threshold of 5%the threshold from 5%

Under the darkness, the mycelia growth varies between 62.5 mm and 73.5 mm, the maximum is obtained on the Czapeck medium with the Dbc2 isolate. We observe a significant difference between the two isolates on the Czapeck medium (69 mm for Dbc1 and 73.2 mm for Dbc2). The Czapeck medium seems to be most favorable to the mycelium growth of the two isolates.

Table 5: Mycelium Growth of the 2 Isolates of H. Bicolor in the Various Synthetic Media with the Darkness

	Culture Media		
Isolats	Czapeck	Richard	Richard's
Dbc1	69a	70a	62.5b
Dbc2	73a	64.8b	63.4b

For the same medium, the values of the same line affected of the same letter do not differ significantly with the threshold from 5%

The mode of alternation light-darkness presented a maximum of growth (79 mm) on the Richard medium for Dbc1 and a minimum on the medium Richard' s (54 mm) for this same isolate. A significant difference is observed between three media used, for the two isolates. The Dbc1 isolate differs significantly from the Dbc2 isolate on the media Czapeck and Richard, the medium Richard' S being less favorable to the growth of the two isolates. The Dbc2 isolate grows better on Czapeck medium than the Dbc1 isolate, whereas this latter believes grows better on the Richard medium.

Table 6: Mycelium Growth of the 2 Isolates of H. Bicolor in the Synthetic Media in Light -Darkness Alternation

	Culture Media		
Isolates	Czapeck	Richard	Richard's
Dbc1	64.2b	79a	54c
Dbc2	72.5a	64.2b	59.3c

For the same medium, the values of the same line affected of the same letter do not differ significantly with the threshold from 5%

Sporulation

In vitro, the sporulation of the isolates (Table 7-8) of *Helminthosporium bicolor* depends on the culture medium used. The comparison of the averages of the number of spores of the levels of treatment carried out revealed significant differences between the complex media and the isolates for the various luminous modes. The rice flour medium has been the most favorable to the sporulation, whether in the darkness, light, or with light-darkness alternation. For the last two modes, the Dbc1 isolate shows a significant difference with the Dbc2 isolate on the rice flour medium of rice. The intensity of sporulation for the isolates varies between 5732.1 and 14011.8 /mm2 spores under the light; between 1273.8 and 3343.72 spores /mm2 under the darkness and of 5095.2 and 9553.5 spores/mm2 under light-darkness alternation. The light stimulates the production of the greatest number of spores for the two isolates considered. We notice the lowest rate of sporulation in the darkness. For the other culture media (PDA, malt Extract, oat Extract) and for a luminous mode; we do not observe a significant difference between the isolates. With light-darkness alternation, we notice a significant difference between the two isolates on PDA medium, on malt Extract and on rice flour medium. On the other hand, in the darkness we notice no significant difference between the isolates. There is no significant difference between light and alternation mode but there is a significant difference between light and darkness mode. We notice the same thing for malt extract medium. With regard to the rice media flour and PDA, the light mode differs significantly from that of the darkness and alternation. In addition, the mode alternation gives significant results different from those obtained in the darkness.

The Dbc1 isolate sporulates better than the Dbc2 isolate on all the media considered and all confused luminous modes. The rice medium flour and PDA medium proves most conducive condition for the formation of the conidia in the light.

Table 7: Average Sporulation (spore/mm ^{2}) of the H. bicolor Isolates of According to the Nature of the and Culture Medium Luminous Treatment

		Mode		
Medium	**Isolates**	**Light**	**Darkness**	**Alternation**
Oat	Dbc1	8279.7a	3343.72b	7642.8a
	Dbc2	8139.58a	2706.82b	7509.05a
Malt	Dbc1	6369a	1592.25b	6209.77a
	Dbc2	5732.1a	1273.8b	5095.2a

Rice flour	Dbc1	14011.8a	1910.7c	9553.5b
	Dbc2	11464.2a	2547.6c	7642.8b
PDA	Dbc1	8916.6a	1910.7b	7642.8b
	Dbc2	8279.7a	2547.6c	5095.2b

For the same medium, the values of the same line affected of the same letter do not differ significantly with the threshold from 5%.

Table 8: Average Sporulation Average (Spores/mm ²) of the 2 Isolates of H. Bicor According to the Luminous Treatment

		Mode		
Media Culture	**Isolates**	**Light**	**Darkness**	**Alternation**
-	Dbc1	7324.35b	1433.02c	9871.19a
Richard	Dbc2	6369a	1910.5b	7324 .35a
Czapeck	Dbc1	12101.1a	3343.72c	10986.52b
	Dbc2	10986.52a	3662.17b	10827.3a
Richard's	Dbc2	7802.02a	1592 .25b	7005.9a
	Dbc2	8916.6a	1910.7c	7483.57b

For the same medium, the values of the same affected line of the same letter do not differ significantly with the threshold from 5%.

On the synthetic media (Table 8), the comparison of the averages of the median number of spores formed by two isolates tested on the same culture medium, highlights significant differences. The Czapeck medium in general induces the greatest number of spores in the light, the darkness and alternation with a maximum of 12101.1 spores /mm ² for Dbc1 in the light. The Czapeck and Richard media favor a more intense formation of spores at the Dbc2 isolate in the light and alternation, whereas the medium Richard's medium favors a better sporulation of Dbc2 in the light, the darkness and alternation.

Influence of pH on the Mycelium Growth and the Sporulation of the Helminthosporium Bicolor Isolates

Influence of the pH

The statistical analyses of the data show that the effect of the interaction pH-isolate is highly significant (Table 9-10). Indeed, for the same pH level of treatment, there is no significant difference between the isolates (Dbc1 and Dbc2). The highest number of spores is obtained from the pH6 for the Dbc1 isolate. Over the whole pH, the number of spores produced varies between 6369 and 10827.3 spores/mm ². For the same isolate and different pH level treatment, there are significant differences between the numbers of produced spores. Thus, for the Dbc 1 isolate, the pH 6 (10827.3 spores/mm ²) and pH7 (9871.94 spores/mm ²) differs significantly from pH4, pH5, pH8 and pH9, whereas for the isolate Dbc2, only the pH6 (4935.37 spores/mm ²) differs significantly from the pH4, the pH5, Ph9. The influence of the pH on the diametrical growth of the mycelium of the two isolates is only partially noticeable since there are no significant differences between the two tested isolates for the same level in pH, but there is, on the other hand, a significant difference between different tested pH for the same level of isolate treatment. The mycelium growth levels vary between 5 and 8.5 cm, the maximum is allotted to the Dbc1 isolate on PH6 and the minimum is recorded with the pH4 and pH9.

Influence of the Temperature on the Mycelium Growth and the Sporulation of the H. Bicolor Isolates

The results of the statistical analyses show the significant differences on the level from the isolates as well as the one of temperatures. The mycelium growth of each isolate considerably varies according to the tested temperatures (Table 11-12). The mycelium growth varies between 1 and 9 cm, the maximum value is observed at 28°C temperature for the Dbc2 isolate (9cm); there is a significant difference between the temperatures of 28.20°C and that of 35.42, 15 and 10°C for the two isolates. The temperatures of 42 and 10°C have caused the lowest mycelium growth. The two isolates are able to grow with 28°, 20°, 35°, 15°C, but could not normally resist the temperatures of 10° and 42°C.

Table 9: Average Mycelial Growth (mm) of the 2 Isolates of H. Bicolor with Regard to the pH

	Isolates	
pH	**Dbc1**	**Dbc2**
4	55a	53.7a
5	58.7a	56.2a
6	85a	77.5
7	77.5a	71.2a
8	61.2a	68.7
9	50a	58.7a

For the same medium, values of the same line affected of same the letter different by significantly to the threshold from 5%

Table 10: Mycelium Growth (mm) of the 2 isolates of H. Bicolor According to the Temperature

	Température (°C)					
Isolates	10	15	20	28	35	42
Dbc1	10^{e}	50c	80a	85,5a	65b	25d
Dbc2	15b	30c	85a	90a	70b	12d

For the same medium, values of the same line affected of the same letter different step significantly with the threshold of 5%

With regard to sporulation (Table 10-11), isolates differ significantly for the temperatures of 10°, 20° and 28°C. The maximum of sporulation is obtained at 20°C (10986,52 /mm2 spores) for the Dbc1 isolate and the minimum at 10° and 15°C (1273,8 /mm2 spores); none of the two isolates could sporulate at the temperature of 42°C. The same isolate, subjected to the tested temperatures, sporulate in a significantly different way. Indeed, there is a significant difference between of 20 and 28°C, 28 and 35°C and 15 and 10°C temperatures.

Table 11: Average Sporulation (/mm2 Spores) of the 2 Isolates of H. Bicolor According to the pH

	Temperature (°C)					
Isolate	10	15	20	28	35	42
Dbc1	0^{e}	1273.8d	10986.52a	8120.47b	2547.6c	0c
Dbc 2	1273.8	1273.8cd	8279.7a	7005.9b	1910.7c	0c

For the same medium, values of the same line affected of the same letter different step significantly with the threshold of 5%

Table 12: Average Sporulation (Spores/mm2) of the 2 Isolates of H. Bicolor According to the Temperature

	Isolates	
pH	Dbc1	Dbc2
4	7005,9a	6840,3a
5	7477,2a	7158,74a
6	10827,3a	9871,94a
7	9871.94a	9069.44a
8	7795.64a	8751a
9	6369a	7477b

For the same medium, values of the same line affected of the same letter different step significantly with the threshold of 5%

DISCUSSIONS

The mycelium growth of the two isolates of *Helminthosporium bicolor* varies according to the tested culture medium. The composition of the culture medium then becomes a great factor for the growth of the fungi species. All the media (complex and synthetic media) allowed the mycelium development of the studied isolates but at different levels. Among the complex media tested, the rice flour and P.D.A media proved to be most favorable for the mycelium growth in the light, the darkness and light-darkness alternation. Similarly, among the synthetic media, Czapeck and Richards media have made it possible to obtain the best mycelium growth for the two isolates in the darkness and at light darkness alternation whereas in the light, the maximum mycelia growth have been produced by Richard and Czapeck media.

Moreover, the sporulation is also influenced by the variation of the culture medium; it is at its optimum level of rice flour and P.D.A media in the light and alternation and on the oats medium in the darkness. According to Mitra (1930), P.D.A medium favors an intense sporulation for *H. hawaiiense, H. tetramera, H. maydis and H. rostratum*. In the same way, Awuah (1989) and Susuri and Hagedorn (1996) concluded that this same nutritive medium induced a better production of spores and a mycelial development of *Curvilaria sp*. and of *Helminthosporium sp*.

On the synthetic media, the Czapeck medium is the most conducive to the sporulation with the continuous light and darkness, whereas the Richard medium is favorable in the alternation. Likewise, Czapeck and Richard induce an intensity of sporulation of *H. hawaiiense, of H. tetramera and H. maydis*, the Richard medium is, on the other hand, unfavourable to the sporulation of *H. turcicum and H. bicolor*.

On the basis of the findings, we notice that the complexes stimulate the formation of the conidies more than the synthetic media, which is in accordance with of Rieuf and Teaska (1973) works which confirmed that the complex media are more conducive than the synthetic media for the growth and the sporulation of several species of *Helminthosporium*.

The temperature has different influences on the various stages of life of H. bicolor. Thus the mycelium growth and the sporulation of the isolates of this species are minimal at 42° and 10° C and optimal at 20° C. According to several scholars (Honda, 1969; Or 1985; Lucas et al. , 1985), the temperatures ranging from 25 to 30 °C represent optimal temperatures for the mycelia growth of *H oryzae*. This confirmation is quite characteristic of the above mentioned species, since in this very case, the optimal sporulation is rather recorded with 20°. The of 28°C temperature is not without producing a big number of spores but its effect remains quite low. Even (Curtis, 1961; Bao et al. , 1993) contended for

example that the temperatures ranging between 15 and 33° C represent optimal temperatures for the growth of the mycelium of *curvularia lunata*.

CONCLUSIONS

Our findings are in accordance with those advanced by Paul and Parbery (1966), who reported that to the temperature of 25°C, several mature ascorspores of *Dreschlera bicolor* appear but their growth remains slow. The best results with mature ascosporea (more than 20 days old) are obtained with 20°C. These same scholars have also contended that the best medium for the sporulation is the saccharose medium - agar – barley flour. They add that the light does not play an influential in the formation of the spores of *H .bicolor*. On this last point, our findings do not confirm this contention. In addition the temperature plays an important part in the growth of an organism. The increase in the temperature causes an increase in enzymatic activity, but when one is located at very high temperatures, the enzymes become inactivated. Moore- Leandeeker (1982) said that the inaptitude of certain mushrooms to develop with a high temperature can be directly related to their incapability to synthesize, at this temperature, substances necessary for the growth, mainly vitamins. Our experiments concerning pH, revealed that the two isolates studied can develop on a wide range of pH from 4 to 9. The maximum mycelium growth is obtained with the pH 6 and 7. Scharndt and Al (1994) have maintained that for certain fungi, the pH range between 5.5 and 8. The two isolates of H. bicolor tested are able of sporulate at various tested PH. The most important conidian fructification is observed with pH of about 6.7 and 8. Moore-landeeker (1982), says that the concentration in hydrogen ion of the culture medium has an impact at two levels: first, it has an impact on the solubility of certain mineral elements, and, second, it influences the permeability of cells, which is modified at certain pH acids or alkaline. A major study of the physiology of pathogenic agent appears to be very useful for a better knowledge of the relation host - parasite. Thus, such a study must be able to lead to a knowledge concerning the pathogenic capacity, varietal resistance (Hau and Rush, 1980) and different methods to use (Dikinson, 1976; Bashi E Fokkennea 1977; Sharma et al., 1989; De Cal et al.; 1993).

REFERENCES

1. **Angladette, A. (1966).** Le Riz : Techniques Agricoles et Production Tropicale. *Agriculture. Agriculture Handbook*, 448. , 16-21.
2. **Awuah, R. T. (1989).** Preparation and Evaluation of Dehydrated Mycological Media from West African Raw Materials. *Annals of Applied Biology*, 19(9): 1-2.
3. **Bao j.r.,Zhan Y.C., Wu X.S & Yu S.F (1993).** Studies of the Pathogen of Stem Rot of Chinese Water chestnut and its Biology. *Phytophylacica Sinica*, 20, 307-311.
4. **Bashi E Fokkema N.J. (1977).** Environmental Factors Limiting Growth of Sporobolomyces Roseus, an Antagonist of Cochliobolus Sativus, on Wheat Leaves. *Trans. Br. Mycol. Soc*., 68, 17-25.
5. **Bean G. and Schwartz R., (1961)**. A Severe Epidemic of *Helminthosporium brown* Spot Disease *Rep*., 45,901.
6. **Benkirane R. (1995).** Contribution à l'Etude des Maladies du Riz au Maroc : Cas de la pyriculariose due à *Pyricularia oryzae*. Thèse Doct.3è Cycle, Univ. Ibn Tofaïl, Fac.sc.Kénitra, Maroc, 189 p.
7. **Benkirane R.,et al.(1995b**) Un Essai pour déterminer la sensibilité ou la résistance des variétés de riz vis-à-vis de quelques isolats marocains de Pyricularia oryzae. *Rev. Rés. Amélior. Prod.agr Milieu Aride*, 7 , 131-143.

8. **Benkirane, R., (2001**). Etude d'une population marocaine de Maganporthe grisea (*Pyricularia grisea*) : Caractérisation, spécificité parasitaire et recherche de source de résistance chez le riz (*Oryzae sativa L.*) à la Pyriculariose. Thèse de Doctorat d'Etat es-sciences, université Ibn Tofail FS Kénitra.

9. **Benkirane, R. et al.** (1994). Study of the composition of the fungi flora associated with the rice seed in Morocco. - *Fifth Arab congr. Plant protect., Fez, Morocco*, 27 November 2nd December, pp 47.

10. **Bennasser L. (1997)** Diagnostique de l'état de l'environnement dans la plaine de Ghrab: suivi de la macro-pollution et ses incidences sur la qualité hydrochimique et biologique du bas Sedou. Thèse de Doctorat d'Etat. Université Ibn Tokai. Faculté des Sciences de Kénitra, 177p.

11. **Curtis R. (1961)**. Some Host Parasite Relationships in the Curvularia disease of Glagilus in Florida. *Plant Disease* reporter, 45, 512-516.

12. **De Cal A., Pascul S. & Melgarejo P., (1993**). Nutritional requirements of antagonists topeach twig blight, Molinilia laxa, *relation to biocontronl. Mycopathogia*, 121: 21-26.

13. **Dichinson C .H. 1976**- Fungi on the aerial surfaces of higher plants. In Dickinson C. H. and Preece T. F. (eds) .*Microbiology of areal plant surfaces*. London: Academic Press .293-234.

14. **Engelhard T., Zasada C. and Prochacki (1972**). Influence of temperature on dermatophytes. *Mycopathologia and mycologia applicata*, 48: 297- 301

15. **Hau F.C et M.C Rush (1980a**) System for inducing sporulation of *Bipolaris oryzae*. *Plant Disease*, 64, n°8: 788-789.

16. **Honda Y. (1969).** Studies on the effect of light on the sporulation of Helminthsporium oryzae. *Bulletin of institute of agricultural Research*, 21, 63-132.

17. **Hsieeh W.H, Snyder W.C. & Smith S.N.(1979)**. Influence of carbon sources, amino acids and water potential on growth and sporulation of *Fusarium moniliforme*. *Phytopathologia*, 69, 602-604.

18. **Kernkamp M.F. ; R.& Woodruff W.C. (1976)** Diseases of cultivated wild rice in Minnesota. Plant *Disease Reporte*., 60 (9), 771-775.

19. **Kohls C.L., Percich J.A (1983)**. Wild rice yield reductions associated with four fungal Brown Spot epidemic. *Phytopathology*, 73:794 (Abstr)

20. **Kohls C.L., Percich J.A.and Hou C.M. (1987).** Wild rice yield losses associated with growth stage specific fungal brown spot epidemies. *Plant Disease*, 71, 419-422.

21. **Lucas G. B, Campbell C. L. & Lucas L. T., (1895)**. Introduction to plant diseases. Identification and management. Departement of plant pathology North Carolina State University Realeigh , North Carolina . *Blast of rice*, pp.189-191.

22. **Mitra , M (1931)**. A comparative study of species and strains of *Helminthosporium* on certain Indian cultivated crops. *Trans. Br. Mycol. Soc.* 15, 254-293.

23. **Moore –Leandeeker E, (1982a)** *Fundamentals of the fungi*. New Jersey: Prentice – Hall, Englewood Cliffs

24. **Moore – Leandeeker, E. (1982 b)** *Fundamental of the fungi*. New Jersey : Prentice – Hall, Englewood Cliffs. 578 p.

25. **Okeke , B., Seigle Murandi, F. Steinman, R. & Sage, L. (1992a).** Investigation on cultural and cellulotyic activity in *Pyricularia oryzae cavara*. *Agronomic*,12: 325-329

26. **Olufaloji, D.B (1992)** Effect of aeration, light and temperature on spore germination and germ tube growth of *curvularia pallescens* Boed.*Cryptogamie*.13, 259-263.

27. **Olufaloji, D.B.(1986b)**. Production of *Curvularia pallescens boedijn* toxins cryptogrammic *Mycol*., 7, 335-342.

28. **Ou, S.H., (1985)**. *Rice diseases*. England: Kew Surrey Commonwealth Mycological Institute, 380 p.

29. **Pandey, S. C. & Shukla, T. M (1978**). Comparative efficiency of different carbon sources in supporting growth and sporulation of six species of *Helminthosporium*. *Indian Phytopathology*, 31:73-75

30. **Paul, A. R. & Parbery, D. G. (1966**) the perfect state of *Helminthosporium bicolor*. Trans. Br. *Mycol. Soc*. 49 (3), 385-386.

31. **Rieuf, P. and Teaska G. (1973**) Etude sur les Helminthosporiums des céréales du Maroc. *Al awamia*, 46, 29-58.

32. **Schrandt, J.K., Davis, R. M. & Nunez, J.J. (1994**). Host range and influence of nutrition, temperature, and pH on growth of *Pytium*, from carrot; *Plant Disease*, 78 (4):335-338.

33. **Sharma R.C., Sharma H.L. & Singh H. (1992**). Effect of incessant rain on seed health and measures to control damage. *International Rice Research Newsletter*, 17:17.

34. **Susuri L. & Hagedorn D. J., (1996**). Sporulation and Disease initiation and development of *Ascochyta pisi* LIB. Pathogenic topea (*Pisum sativum L.)* Zbomik-Biotechniske fakultate Univerze V Ljublani (Slovenia) Kmetijstvo 67, 147-150.