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# Effects of Temperature and pH on Mycelium Growth of *Phoma sorghina* (Sacc.) Boerema Dorenbosch and Van Kesteren *in vitro*

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**Abstract:** The effects of temperatures 22, 28, 32, 36 and 40°C and those of pH 5, 6.5 and 6 were evaluated on 11 isolates of *P. sorghina* on malt agar medium. The optimal mycelium growth of the most isolates is noted at 28°C. At 32°C, we have recorded a significant reduction of mycelium growth of all the isolates tested when compared with the control at 22°C. At this same temperature, *P. sorghina* isolates can be group on sensitive isolates, on moderately isolates and on resistant isolates to temperature. The mycelium growth of all the isolates is inhibited at 36°C. On the other hand, the temperature of 40°C kills the mycelium of all the isolates of *P. sorghina*. The results of our work also show that, least variation of pH (6.5-6) significantly reduced the mycelium growth of *P. sorghina* isolates at 22 and 28°C. At pH 5 most of the isolates tested are well adapted and the mycelium growth is more important when compare with that at pH 6.

**Key words:** Temperature, pH, mycelium growth, isolates, *Phoma sorghina*

## INTRODUCTION

The development of plants, likewise those of plants pathogens in some environment, is subjected to abiotic and biotic factors. Lacroix (1998) shows that temperature rise affects fungi of tomato root disease development (*Rhizoctonia solani* Kühn, *Fusarium oxysporum* Schlect. Emend. Snyder and Hansen, *Colletotrichum coccodes* (Wallr) Hughes and *Pyrenochaeta lycopersici* R.W. Scheid. and Gerlach). Infection by *Pyrenochaeta lycopersici* is severe in low temperature 13.4°C) but, that of *F. oxysporum* and *C. coccodes* became important when temperature exceed 20°C. He also indicated that most of root rots fungi growth very well when environment is acid. El Abdellaoui *et al.* (2005), show that temperature and pH influence radial growth and sporulation of *Curvularia tuberculata* (Wakker) Boedijn. Effects of temperature and pH on the growth of sol microorganism, is also shown by Taziebou *et al.* (2004). The works of Mensah (2000) and Dillard (1988) pointed out that pH and temperature affect *C coccodes. Metarrhizium flavor viride* and *M. anisopliae* conidia germination.

*Phoma sorghina* is one of fungi responsible of sorghum seed mould (Zida *et al.*, 2010). Evaluation of mycoflora in sorghum seed collected from all the ecological zone of Burkina Faso indicates that. *P. sorghina* infects all the sorghum samples. The infection level can reach 99.5%. As regard to sorghum seed infection level by *P. sorghina,* we may logically found problems of germination and mortality after emergence of sorghum plant (Zainun and Parbery, 1974; Purnithalingam, 1985; Porello and Moreno, 2005). But, in spite of sorghum seed infection by *P. sorghina*, in farmer field we do not observe these problems. After seedling, sorghum seed germinate very well. This observation supposes existence of environment factors or microorganism like bacteria and some fungi that influence the development of *P. sorghina* in the sol in our country. Environment factors especially pH and sol temperature influence the growth of micro-organism. As a matter of fact, the diversity of micro-organism in sol is according climate zone.

The objective of this study is to shown the effects of pH and temperature on the mycelium growth of *P. sorghina in vitro*. This study is necessary to understand the ecological of *P. sorghina* in Burkina Faso.

## MATERIALS AND METHODS

**Material:** For this study, eleven isolates of *P. sorghina* collected from sorghum seed, rice seed, millet, weeds and one reference isolate (181.80) are use. The isolates are choosing according to their morphological character on mal agar and their geographic origin.

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## Methods

**Medium preparation:** Medium is prepared by mixing 20 g of agar and 20 g of malt in 1000 mL of water. The temperature of mix is raised at 100°C to melt agar and malt. The mix obtained is cooled at 60°C and the pH of the mix is adjusted by introducing little quantity of concentrate chlorhydric acid in the mix and the pH is measured using a pH-meter. The operation is repeated for obtaining the pH wanted. The mix is sterilized at 120°C for 30 min then it is cooled at 60°C. After that, the medium is aseptically divides in Petri dishes of 9 cm of diameter. The control medium is obtained by mixing suitable quantities of agar and malt in proper quantity of water. The mix is sterilized in the same conditions. After cooling, the medium is aseptically divided into Petri dishes of 9 cm of diameter.

**Experimental device:** The experimental device study is a split-plot with two blocs. Each bloc is composes by eleven (11) treatments repeated in three time. The factors studies are pH (pH 5, 6 and control that pH is 6.5) and temperature (22, 28, 32, 36 and 40°C).

**Inoculation:** In the speed growing zone of each fungus isolate of five days old, a mycelium explantat of 5 mm of diameter is done with a punch. Each mycelium slice of *P. sorghina* is introduced in the middle of new Petri dish containing mal agar medium at different pH. The Petri dishes are incubated according the conditions of temperature. The first group is incubated at 22°C under 12 h alternating cycle of near ultra violet light and darkness for 7 days. The four others are incubated respectively at 28, 32, 36 and 40°C in darkness for 7 days.

**Evaluation and data analysis:** Evaluation is consisted to measure the mycelium growth of *P. sorghina* species in different temperatures and pH value. For that, two perpendicular lines are made on the cover of Petri dish. These lines are used to measure the means diameter of mycelium growth of each isolate of *P. sorghina* after 7 days.

The data are recorded on Microsoft excel and analysis with SPSS 16.0. Means are compared using the multiples classification of Student Newman and Keuls at the level of 5% when there is significant difference between the treatments. The results are recorded on tables and Figures.

## RESULTS

**Effect of temperature on the mycelium growth of 10 isolates of *P. sorghina*:** Data analysis shows a significant effect of temperature on the mycelium growth of *P. sorghina* isolates. At seven (07) days after incubation, analysis reveals that temperature of 36°C and 40°C inhibit the mycelium growth of all the isolates of *P. sorghina* tested. The temperature of 36°C inhibits the mycelium growth of all the isolates while, at the temperature of 40°C, the mycelium on the slice of medium die. At the temperature of 32°C we recorded significant reduction of mycelium growth for all the isolates compared to the control. For the most of *P. sorghina* isolates tested, the temperature of 28°C favors the mycelium growth when compared with the others temperatures studied. At 32°C *P. sorghina* isolates can be grouping according to their sensitivity to temperature: sensitive isolates to temperature are 1666So11-74(2), 1341So07-65, 1Dh08-1(3), 591Mi06-4, 474So07-40, 576So06-13, moderately sensitive isolates to temperature are 14Da08-10-14(6), 2Spf08-02., 181.80 and the resistant isolates to temperature are 803Ri06-03 and 438So07-39 (Fig. 1).

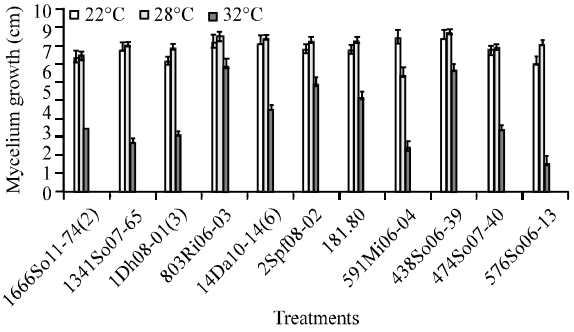


Fig. 1: Effect incubation temperature on the mycelium growth of *Phoma sorghina* isolates after seven days incubation

**Compared growth of *P. sorghina* isolates on different temperatures:** Data analysis revealed a significant difference between *P. sorghina* isolates at the same temperature. At 28°C favor temperature for the mycelium growth of the most isolates. the *P. sorghina* isolates can be classified according to their growth speed on malt agar. So, we distinguish the very speed isolates 803Ri06-03, 438So07-39; the speed isolates 14Da10-14(6), 2Spf08-02., 181.80; the moderated speed isolates 474So07-40, 1Dh08-01(3), 576So06-13, 1341So07-65 and the isolates of slow mycelium growth 591Mi06-04 and 1666So10-74(2) (Table 1).

**Effects of temperature and pH on the mycelium growth of *P. sorghina* isolates:** At the same temperature there is significant difference between the pH tested (Fig. 2). On temperature 22 and 28°C the variable of pH induce significant reduction of mycelium growth between *P. sorghina* isolates excepted the isolates 1Dh08-1(3), 576So06-13, 591Mi06-04 which have important mycelium growth at pH 5 respectively at 22°C and 28°C. The results also reveal that most of *P. sorghina* isolates are sensitive to little variation of pH (Fig. 2-4). When the temperature

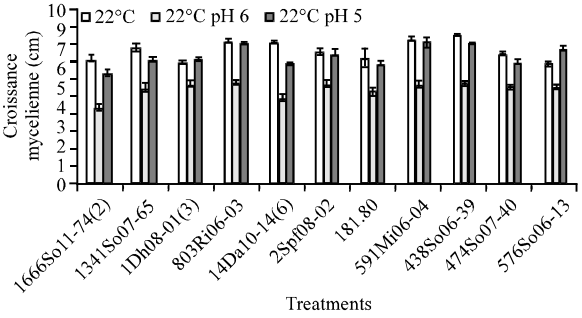


Fig. 2: Effect of temperature and pH on mycelium growth of *Phoma sorghina* at 22°C

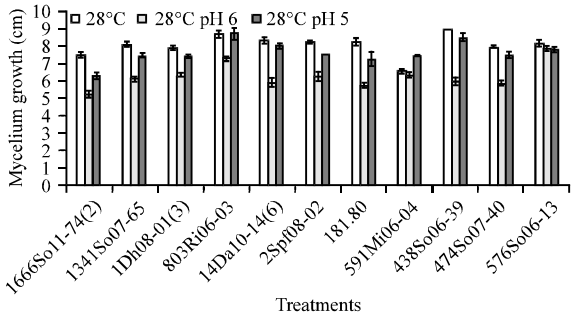


Fig. 3: Effect of Temperature and pH on mycelium growth of *Phoma sorghina* at 28°C

Table 1: Comparison of effect of different temperatures on mycelium growth of *Phoma sorghina* isolates at 7 days after incubation

| *Phoma sorghina* isolates | Temperature (°C) | | | | |
|---|---|---|---|---|---|
| | 22 | 28 | 32 | 36 | 40 |
| 1666So11-74 (2) | 7.53ab | 7.53b | 3.43c | 0 | 0 |
| 1341So07-65 | 8.03cd | 8.13cd | 2.73b | 0 | 0 |
| 1Dh08-01(3) | 7.3a | 7.93c | 3.16c | 0 | 0 |
| 803Rio6-03 | 8.56ef | 8.7e | 7.03g | 0 | 0 |
| 14Da10-14(6) | 8.43de | 8.36d | 4.7d | 0 | 0 |
| 2Spf08-02 | 7.93bc | 8.26d | 5.9f | 0 | 0 |
| 181.80 | 7.56ab | 8.26d | 5.1e | 0 | 0 |
| 591Mi06-04 | 8.86f | 6.56a | 2.6b | 0 | 0 |
| 438So07-39 | 8.96f | 9f | 6.93g | 0 | 0 |
| 474So07-40 | 7.93bc | 7.96c | 3.4c | 0 | 0 |
| 576So06-13 | 7.2a | 8.2cd | 1.7a | 0 | 0 |
| F-valeu | 19.85 | 56.09 | 180.76 | - | - |
| Probability | 0.000 | 0.000 | 0.000 | - | - |
| Significant | THS | THS | THS | - | - |

So: Sorghum, Dh: *Digitaria horizontalis*, Ri: Rice, Da: *Dactylotenum aegyptium*, Spf: *Setaria pallid-fusca*, Mi: Millet, THS: Very highly significant: No discrimination between treatments. The means followed by the same letter in the same colon are not significant different at the level at 5% according to multiples classification of Student Newman and Keuls

rise to 32°C the isolates 1Dh08-01(3), 591Mi06-04, 4So07-40 and 576So06-13 became little sensitive to pH variation. At pH 5 most of isolates come to put up with pH and temperature, as well as we notice increasing of mycelium growth compared with pH 6 excepted, isolate 803Ri06-03 which have low mycelium growth at pH 5

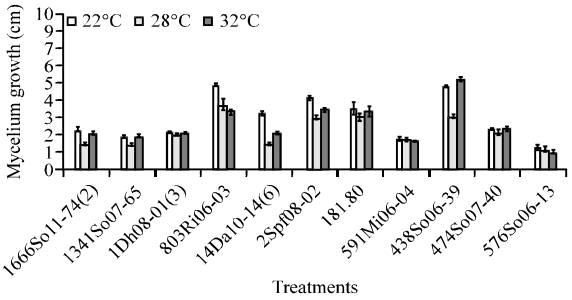


Fig. 4: Effect of Temperature and pH on mycelium growth of *Phoma sorghina* at 32°C

compared with pH 6 at 32°C (Picture 3). At pH 5 the mycelium growth of isolate 576So06-13 is stimulated at 22°C with comparison to the control (Fig. 2).

## DISCUSSION

Temperature and pH are among the environment factors that influence the development of micro-organism in sol (Taziebou *et al*., 2004; Kaiser *et al*., 2005; Inam-Ul-Haq *et al*., 2009). The effects of these factors are evaluated on the mycelium growth of *P. sorghina* isolates *in vitro*. The results indicated that temperature and pH separately influence the mycelium growth of *P. sorghina* isolates. The results of many works point out that temperature and pH influence negatively the development of fungi (Dillard, 1988; Sparringa *et al*., 2002; Begoude *et al*., 2007 Montealegre *et al*., 2009; Li *et al*., 2010). The optimal mycelium growth temperature is 28°C. For all the isolates of *P. sorghina* tested, the temperature of 36°C inhibits the mycelium growth, which of 40°C kills the fungus. In Burkina Faso, sorghum seedling takes place at the period of June to July 15th. This period in Burkina Faso is characterized by high temperature which can reach 40°C. The rising of sol temperature up to 36°C could inhibit the mycelium growth or kill the fungus. The elimination of *P. sorghina* by hot on sorghum seed can contribute to improve their germination. The results of study also reveal that little variation of pH between 6.5 and 6 reduce significantly the mycelium growth of most isolates of *P. sorghina*. On the other hand, at pH 5 the mycelium growth of *P. sorghina* isolates tested is important to comparison with *P. sorghina* isolates growth at pH 6. Lacroix (1998) showed that fungi responsible of root rot grow well on acid medium.

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