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TEMPERATURE AND PH IMPACT ON THE MYCELIUM GROWTH OF *MYCOGONE PERNICIOSA* AND *VERTICILLIUM FUNGICOLA* ISOLATES DERIVED FROM POLISH AND FOREIGN MUSHROOM GROWING HOUSES

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Abstract: The research project aimed at determining the effect of incubation temperature and pH of the agar medium on the mycelium growth of *Mycogone perniciosa* (Magnus) Delacroix and *Verticillium fungicola* (Preuss) Hassebrauk in *in vitro* conditions. Ten *M. perniciosa* and ten *V. fungicola* isolates were applied. Mycelium growth of the above-mentioned isolates was studied on PDA agar medium (Merck) enriched with an extract from mushroom hyphae, at temperatures ranging from 15 to 30°C and pH from 5.5 to 7.5. Incubation temperature was found to affect the mycelium growth rate of the examined pathogens. Mycelium growth in all *M. perniciosa* and *V. fungicola* isolates was the fastest at the temperature of 25°C, while a temperature of 15°C inhibited their growth significantly. Mycelium growth of the examined pathogens also depended on medium pH. *M. perniciosa* mycelium developed best at pH = 5.5, whereas at the same medium pH *V. fungicola* mycelium exhibited the worst growth.

Key words: *Mycogone perniciosa*, *Verticillium fungicola*, temperature, pH, mycelium growth

INTRODUCTION

Wet bubble and dry bubble, caused by the *Mycogone perniciosa* (Magnus) Delacroix and *Verticillium fungicola* (Preuss) Hassebrauk mycoparasites, are diseases associated with *Agaricus bisporus* (Lange) Imbach cultivation (Umar *et al*. 2000; Sharma and Singh 2003). For some time now, the name *V. fungicola* has been used interchangeably with the name *Lecanicillium fungicola* (Preuss) Zare&Gams (Zare and Gams 2008). The above-mentioned pathogens occur in many regions of the world and losses caused by them in *A. bisporus* cultivations vary considerably (Gea *et al*. 2003, Maszkiewicz *et al*. 2006; Glamoclija *et al*. 2008; Meyer and Korsten 2008). In Poland, both diseases occur quite frequently, especially in small mushroom growing farms. The frequency of the diseases is mainly due to considerable production fragmentation as well as inappropriate hygiene conditions. The development of pathogens, their virulence and spreading velocity, to a considerable extent depend on conditions prevailing in the cultivation facilities. These conditions are, among others, temperature and cover pH (Maszkiewicz *et al*. 2006; Fletcher and Gaze 2008).

The objective of the investigations was to determine the impact of the incubation temperature and agar medium pH on mycelium growth of several *M. perniciosa* and *V. fungicola* isolates obtained from domestic and foreign mushroom growing houses.

MATERIALS AND METHODS

The following isolates of pathogenic fungi were used in the described investigations:

– *M. perniciosa* – PGM5, PGM6, PGM8, PGM9, PGM15, PGM16 and PGM20 derived from the collection of the Institute of Horticulture in Skierniewice, Poland, and MP10/2, MP12/4 and MP22/14 derived from the collection of the University of Life Sciences in Poznań, Poland
– *V. fungicola* – 16A, 23A, V25, V26 and V31, which derived from the collection of the Institute of Horticulture in Skierniewice, Poland and V4/1, V28/11, VHL1, VHL2 and VHI1 derived from the collection of the University of Life Sciences in Poznań, Poland.

The examined isolates were obtained from *A. bisporus* cultivations situated in different regions of Poland with the exception of *V. fungicola* VHL1 and VHL2 isolated from infected carpophores derived from Holland and VHI1 derived from Spain.

Experiments were carried out on sterile Petri dishes of 9 cm diameter on a PDA medium (Merck, pH = 6.6) enriched with extract from *A. bisporus*. The extract was obtained by submerging 200 g carpophores in 500 cm^3 distilled water and then breaking them up in a mixer. The obtained material was dried on a filter paper and the obtained filtrate was added to the substrate. Each 1 dm^3 substrate was enriched with a 20 cm^3 extract. Following

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a 15-minute sterilisation at a temperature of 121°C, the liquid medium was poured onto Petri dishes. When the medium solidified, it was inoculated with 4 mm in diameter agar discs which were overgrown with the mycelium of pathogenic fungi. The discs were placed in the centre of the dish, in such a way that the mycelium adhered directly to the medium surface.

Two separate experiments in a completely random design, in five replications and two series were established. In the first trial, the impact of incubation temperature on the growth of pathogenic fungi was examined. The following incubation temperatures were applied: 15, 20, 25 and 30°C. In the second trial, media of varying pH were employed. The following pH values were applied: 5.5, 6.0, 6.5, 7.0 and 7.5. Medium pH was regulated using 1% citric acid and 0.1 n NaOH. In this case, the incubation was carried out at a temperature of 25°C. In both experiments, measurements of the mycelium colony diameter were taken after 21 days of incubation.

The assessment of the investigation results was performed with the assistance of the analysis of variance for factorial experiments at $\alpha = 0.05$ by the Keuls-Newman test.

RESULTS AND DISCUSSION

Incubation temperature was found to affect mycelium growth of both examined pathogens (Figs. 1, 2). The mycelium of all the examined *V. fungicola* isolates developed best at a temperature of 25°C, poorer at a temperature of 20°C, and its worst growth was recorded at 15°C. An incubation temperature of 30°C caused total mycelium growth inhibition of all the tested isolates, with the exception of isolate VHI1. The response of all *V. fungicola* isolates to the incubation temperature was similar with only slight deviations, and with the exception of isolate VHI1. The mycelium of this isolate continued to grow even at the temperature of 30°C, whereas temperatures of 20 and 15°C clearly reduced the growth rate of the mycelium in comparison with the remaining isolates. Geels *et al*. (1988) reported that temperatures above 20°C strongly accelerated infestation of the cultivation with the pathogen. Gams and Zaayen (1982) as well as Zaayen and Gams (1982) emphasised the fact that two forms of *V. fungicola* can occur in the cultivation of *A. bisporus*, differing from each other by the optimal temperature of mycelium growth. In the case of *V. fungicola* var. *fungicola*, the optimal temperature of mycelium growth ranges from 20 to

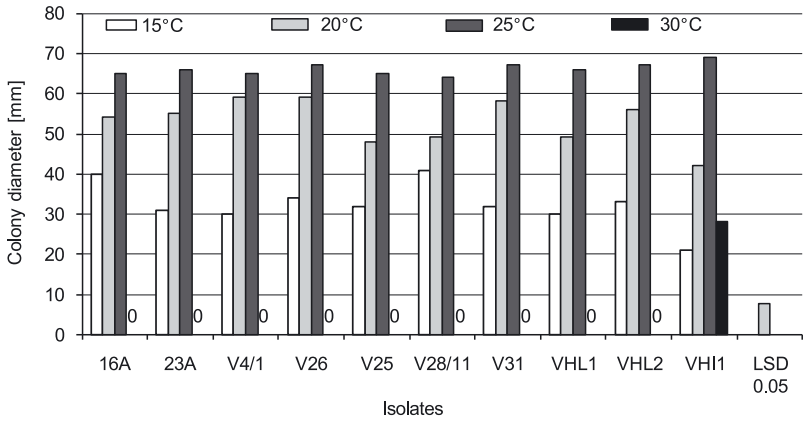

Fig. 1. Influence of temperature on the mycelium growth of some *V. fungicola* isolates

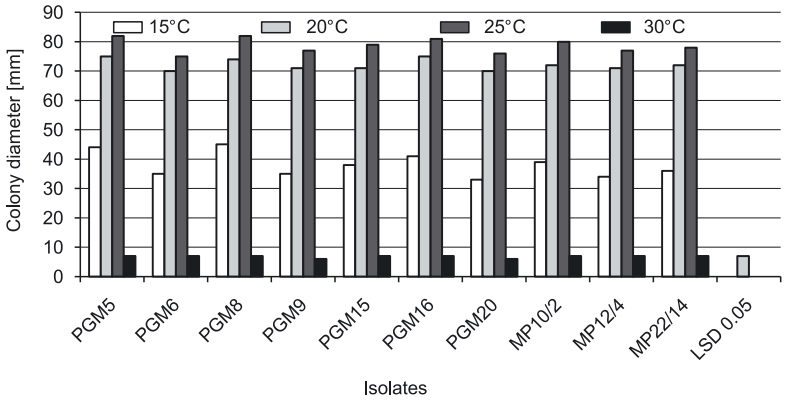

Fig. 2. Influence of temperature on the mycelium growth of some *M. perniciosa* isolates

24°C, whereas for the *V. fungicola* var. *aleophilum* – from 24 to 27°C. Both the above forms are very similar morphologically and differences between them are, primarily, of a physiological nature. It appears that all of the *V. fungicola* isolates tested in our experiments belonged to the first of the above-mentioned forms, and the optimal temperature of their mycelium growth was close to that quoted by the above researchers, whereas the VHI1 isolate exhibited traits of the *V. fungicola* var. *aleophilum*. Juarez del Carmen *et al*. (2002) demonstrated that isolates of *V. fungicola* var. *fungicola* and *V. fungicola* var. *aleophilum* grew similarly at a temperature of 23°C, while at a temperature of 30°C, they recorded growth only in the case of the *V. fungicola* var. *aleophilum* mycelium. The membership of the examined isolates from our experiments to the above-mentioned forms could only be confirmed by detailed morphological and molecular studies (*e.g.* PCR). In the case of all the examined *M. perniciosa* isolates, their mycelia developed best at a temperature of 25°C and only slightly worse at the temperature of 20°C. On the other hand, the applied temperature of 15°C slowed down the rate of mycelium growth, significantly. This corroborates information given by Lambert (1930) who claimed that a temperature of 24°C is optimal for the growth of this pathogen. Glamoclija *et al*. (2008) conducted studies on the morphology of different *M. perniciosa* isolates and considered 25°C as the temperature optimal for their development. Also Tan *et al*. (1994) concluded that the above temperature was optimal for the growth of *M. perniciosa* mycelium. An incubation temperature of 30°C was found to considerably reduce mycelium development of all the examined *M. perniciosa* isolates. It appears that in the case of occurrence in the cultivation of both of the discussed diseases, their development could be checked by reducing temperature to 15°C or lower. Nair and Macauley (1987) found that decreasing temperature from 20°C to 14°C decreased the development of *V. fungicola*. On the other hand, Bech *et al*. (1989) reported that in France, in the situation of mushroom cultivation in underground bunkers where the temperature amounted to 15°C, diseases caused by *M. perniciosa* and *V. fungicola* occurred much less frequently than in traditional cultivation at the temperature of 16–19°C.

Medium pH was found to affect the mycelium growth of both the examined pathogens (Figs. 3, 4). Growth of the *V. fungicola* mycelium was similar within the entire range

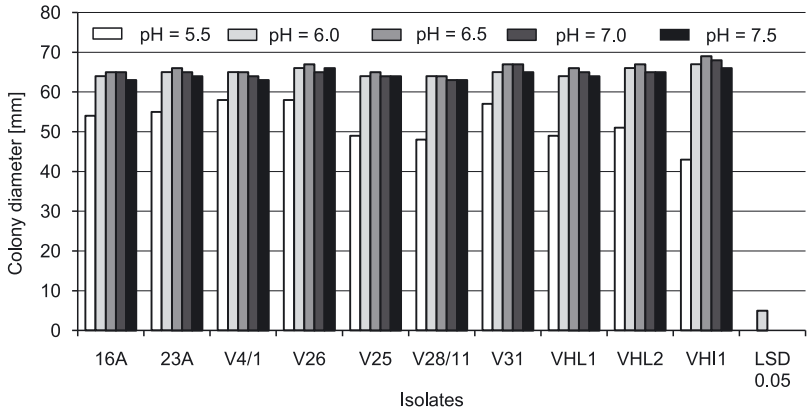

Fig. 3. Influence of medium pH on the mycelium growth of some *V. fungicola* isolates

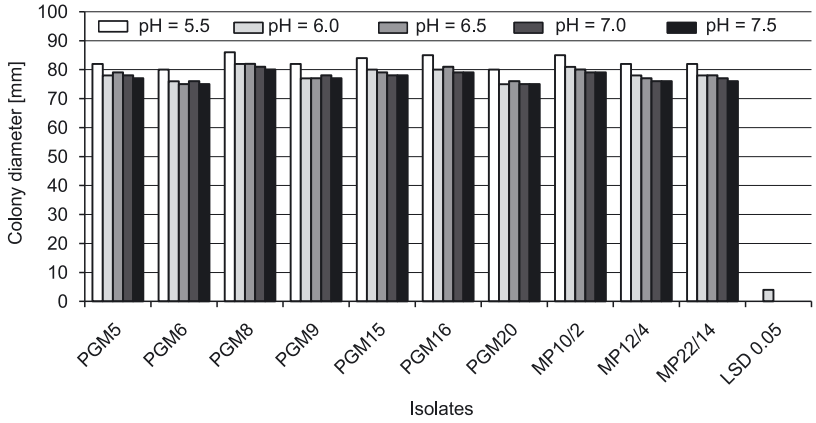

Fig. 4. Influence of medium pH on the mycelium growth of some *M. perniciosa* isolates

of the applied pH values of the medium, with no apparent differences between the experimental isolates. The only exception was pH = 5.5, where the mycelium was found to grow worse than in the case of the remaining reaction values. The authors failed to find in the available literature, data about optimal pH values for *V. fungicola* mycelium growth. Thapa and Jandaik (1986) reported that sprouting of *V. fungicola* spores was best at pH values ranging from 5.0 to 5.5. In addition, the above researchers also found that within the above pH rate, the sprouting hyphae were characterised by the greatest length. It is difficult to account for the discrepancies between the results of our research and those of the above-mentioned researchers. In the case of *M. perniciosa*, mycelium developed best at pH = 5.5, irrespective of the examined isolate. Within the remaining range of the medium pH values, the mycelia of all isolates developed at similar rates. The obtained results confirm the findings of Tan *et al*. (1994), that the optimal pH for the development of *M. perniciosa* mycelium ranges from 5 to 5.6.

CONCLUSIONS

1. Incubation temperature influenced mycelium growth of the examined *M. perniciosa* and *V. fungicola* isolates. The temperature most favourable for the mycelium development of both pathogens was 25°C, while a temperature of 15°C checked their growth significantly.
2. The VHI1 isolate of *V. fungicola* showed high resistance to the incubation temperature of 30°C.
3. Within the examined range of the medium pH, pH = 5.5 was found to be the most favourable for the mycelium development of the examined isolates of *M. perniciosa*, and limited the growth of *V. fungicola* isolates.

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POLISH SUMMARY

WPŁYW TEMPERATURY I PH NA WZROST GRZYBNI IZOLATÓW *MYCOGONE PERNICIOSA* I *VERTICILLIUM FUNGICOLA* POCHODZĄCYCH Z POLSKICH I ZAGRANICZNYCH PIECZARKARNI

Badania miały na celu określenie wpływu temperatury inkubacji oraz odczynu pożywki agarowej na wzrost grzybni *Mycogone perniciosa* (Magnus) Delacroix i *Verticillium fungicola* (Preuss) Hassebrauk, w warunkach *in vitro*. Wykorzystano 10 izolatów *M. perniciosa* pozyskanych z pieczarkarni, z różnych rejonów Polski (8 izolatów) oraz Niemiec (2 izolaty), a także 10 izolatów *V. fungicola* (7 krajowych i 3 z terenu Hiszpanii i Holandii). Wzrost grzybni wymienionych izolatów badano na pożywce agarowej PDA (Merck) wzbogaconej wyciągiem z owocników pieczarki w temperaturze od 15 do 30°C (co 5°C) i pH od 5,5 do 7,5 (co 0,5 pH). Stwierdzono wpływ temperatury inkubacji na szybkość wzrostu grzybni badanych patogenów. Wzrost grzybni u wszystkich izolatów *M. preniciosa* i *V.*

fungicola był najszybszy w temperaturze 25°C. Odporność *V. fungicola* na temperaturę 30°C była różna dla izolatów pochodzących z Polski i Holandii oraz dla jednego izolatu z Hiszpanii. Temperatura 15°C znacząco ograniczała wzrost wszystkich badanych izolatów *M. perniciosa* i *V. fungicola*. Wzrost grzybni badanych patogenów zależał od odczynu pożywki. Grzybnia *M. perniciosa* rosła najlepiej przy pH = 5,5, a wyższe wartości powodowały nieznaczne zmniejszenie szybkości wzrostu u wszystkich badanych izolatów. W przypadku *V. fungicola* słabszy wzrost grzybni stwierdzono tylko przy pH pożywki wynoszącym 5,5.