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GROWTH OF AGGRESSIVE ISOLATES OF *TRICHODERMA AGGRESSIVUM* F. *EUROPAEUM* IN DEPENDENCE ON TEMPERATURE AND MEDIUM

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Abstract

The effect of temperature and medium on the aggressive *Trichoderma aggressivum* f. *europaeum* isolates mycelium growth was investigated. Two isolates of the species from mushroom-growing houses in Western Europe and 20 isolates from Polish mushroom-growing houses and oyster mushroom cultivations were taken into consideration. Growth of experimental isolates was examined on standard agar medium and on manure medium at 15, 20, 25, 30 and 35°C. The mycelium of the examined isolates grew better on the manure medium than on standard agar medium and the optimum temperature for all the examined isolates was 25 and 30°C.

Key words: *Trichoderma*, isolate, mycelium growth, agar medium, temperature

Introduction

As late as at the beginning of 1980s, according to a number of researchers, green *Trichoderma* moulds did not have a significant impact on mushroom production in Western Europe (Hayes 1978, Gandy 1985, Sharma et al. 1999, Samuels et al. 2002, Fletcher and Gase 2008). Beyer et al. (1997) maintain that it was not until the beginning of 1990s that a new strain of *Trichoderma* responsible for the epidemic of green moulds in mushroom cultivation – *Trichoderma harzianum*, a biotype designated as Th4, was discovered in Pennsylvania. It affects rapidly mushrooms and shows exceptionally high expansiveness. In addition, it also causes considerable losses in cultivations of oyster mushroom and shiitake (Cooke and Rayner 1984, Benhamou and Chet 1993, Beyer et al. 1997).

First reports about the green mould appearance in mushroom cultivations in Poland date back to 2002 (Szczech et al. 2008). The authors showed that the most

frequently isolated species of the *Trichoderma* genus identified in mushroom producing farms included: *T. harzianum*, *T. atroviride*, *T. aggressivum* and *T. longibrachiatum*. In addition, using PCR, multiplex PCR and RAPD techniques, they also found out that the 24 examined isolates (deriving from Polish mushroom-growing houses) belonged to the *T. aggressivum* Th2 biotype.

Samuels et al. (2002) investigated mycelium growth of various *Trichoderma* green moulds isolates and demonstrated that only *T. harzianum* grew well and sporulated at the temperature of 35°C, whereas *T. atroviride* displayed slower growth under these conditions. On the basis of micromorphological studies, the authors classified *T. harzianum* biotypes Th2 and Th4, occurring in mushroom cultivation, as *T. aggressivum* f. *europaeum* and *T. aggressivum* f. *aggressivum*, respectively. In addition, they also found that *T. aggressivum* f. *aggressivum* (Th4) and *T. aggressivum* f. *europaeum* (Th2) failed to show significant morphological differences, although they did show a slight difference in the rate of growth at the temperature of 25°C as well as statistically significant micromorphological differences.

The aim of the performed experiments was to determine the impact of temperature and medium type on the mycelium growth of 22 isolates of *T. aggressivum* f. *europaeum*.

Material and methods

The experiment was carried out in the laboratory of the Department of Vegetable Crops of the Poznań University of Life Sciences.

The following fungal isolates were employed in the experiments: two isolates of *T. aggressivum* f. *europaeum* deriving from mushroom-growing houses from Western Europe – CPK 361 from Austria (University of Technology, Institute of Chemical Engineering, Division of Applied Biochemistry and Gene Technology, Vienna) and CBS 100526 from The Netherlands (Centraalbureau voor Schimmelcultures, Utrecht) as well as 20 isolates deriving from Polish mushroom-growing houses and oyster mushroom cultivations collected at the gene bank of the Department of Vegetable Crops (Poznań University of Life Sciences). The mushroom cultivations were located in many regions of Poland.

Mycelium growth was studied on two agar media: a standard mineral medium and an organic manure medium. The standard medium contained 1.0 g of KH_2PO_4 , 1.0 g of NH_4NO_3 , 0.5 g of $\text{MgSO}_4 \times 7\text{H}_2\text{O}$, 3.0 g of sucrose, 2.0 g of glucose, 1.0 g of maltose and 20 g of agar in 1 dm³ of distilled water. Manure medium was prepared on extract of pasteurized manure. The extract was obtained by boiling 50 g of manure in 1 dm³ of distilled water for 30 min. After manure was strained on a sieve, 22 g of agar was added and distilled water was completed to the content of 1 dm³. The media were sterilized in an autoclave at the temperature of 121°C for 30 min and next they were poured (0.02 dm³) onto sterile Petri dishes 9 cm in diameter. Solidified media were inoculated with 4 mm discs of agar medium overgrown by six-day-old pure cultures of *T. aggressivum* f. *europaeum* isolates. The mycelium

growth took place in the dark in a thermostatic chamber at the temperature of 15, 20, 25, 30 and 35°C and relative air humidity of 85–90%. The rate of mycelium growth was estimated by the diameter of colonies after three days of incubation.

The experiment was established in a completely randomized design in five replications (plates) and two series. The results were analyzed using analysis of variance for two-factor experiments at the level of significance of $\alpha = 0.05$. The results were discussed on the basis of mean values obtained from the two series of experiments.

Results

The examined isolates of *T. aggressivum f. europaeum* (both Polish and those from Western Europe) exhibited differences in their growth at the applied incubation temperatures. The best mean growth on the manure medium showed T24/A (46 mm) and TS13 (45 mm) isolates (Table 1). It was also demonstrated that some of the examined isolates grew distinctly slower on the manure medium. The isolates which were found to grow slowest were: T90/5 (35 mm) and T17/S (36 mm). The Austrian and Dutch strains from mushroom-growing houses from Western Europe exhibited after three days of incubation mean growth rates of 41 and 42 mm, respectively, and did not differ significantly from each other. Mycelium growth of all the examined *T. aggressivum f. europaeum* isolates on the manure medium depended very strongly on the temperature. The best growth was recorded at 25 and 30°C (Fig. 1). At the above mentioned temperatures, the cultures reached the diameter of 54 and 55 mm, respectively, whereas at all the remaining temperatures mycelium growth was significantly weaker. At the temperature of 20°C, the diameter reached 32 mm, whereas at the temperature of 15°C – 13 mm and at 35°C – 6 mm, i.e. there was no growth at this temperature, practically.

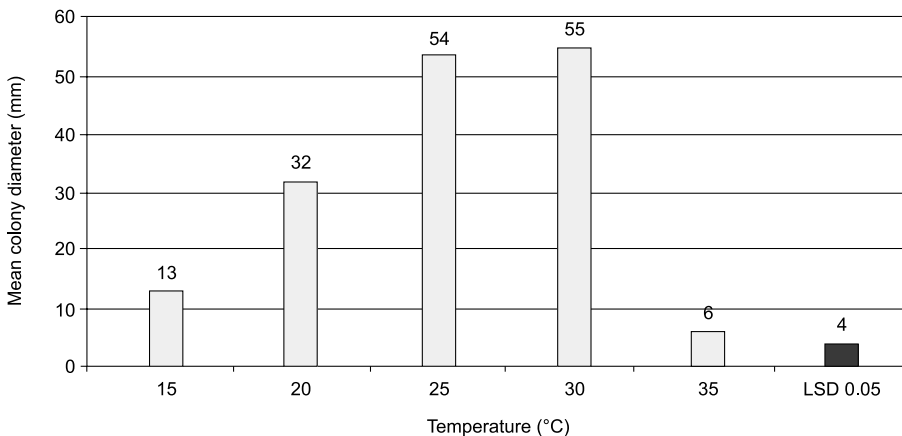


Fig. 1. Mean colony diameter of 22 *Trichoderma aggressivum f. europaeum* isolates after three days of growth on manure medium in relation to temperature (mm)

Table 1

Colony diameter of *Trichoderma aggressivum* f. *europaeum* isolates after three days of growth on manure medium (mm)

| Isolate | Temperature (°C) | | | | | Mean |
|------------|------------------|----|----|----|----|------|
| | 15 | 20 | 25 | 30 | 35 | |
| CPK 361 | 14 | 32 | 54 | 57 | 7 | 41 |
| CBS 100526 | 16 | 35 | 55 | 55 | 6 | 42 |
| T7/23 | 12 | 30 | 53 | 56 | 6 | 39 |
| T9/25 | 11 | 34 | 50 | 51 | 5 | 38 |
| TM/11 | 15 | 33 | 52 | 50 | 7 | 39 |
| TS/13 | 16 | 37 | 58 | 59 | 8 | 45 |
| TA/12 | 14 | 30 | 51 | 53 | 6 | 39 |
| TR/7 | 8 | 33 | 53 | 55 | 5 | 39 |
| T12/4 | 6 | 36 | 56 | 58 | 6 | 41 |
| T17/3 | 15 | 32 | 57 | 56 | 8 | 42 |
| TX/32 | 12 | 33 | 55 | 54 | 6 | 40 |
| T31/1 | 14 | 35 | 54 | 54 | 5 | 41 |
| T40/5 | 13 | 32 | 56 | 58 | 6 | 41 |
| T17/S | 9 | 27 | 50 | 53 | 6 | 36 |
| T24/A | 17 | 38 | 58 | 62 | 7 | 46 |
| T30/B | 16 | 32 | 54 | 60 | 5 | 42 |
| T11/8 | 10 | 30 | 52 | 56 | 6 | 39 |
| T75/8 | 13 | 34 | 55 | 57 | 7 | 42 |
| T81/2 | 8 | 30 | 56 | 59 | 8 | 40 |
| T90/5 | 9 | 29 | 48 | 46 | 7 | 35 |
| T93/7 | 15 | 32 | 53 | 55 | 6 | 40 |
| T97/2 | 14 | 30 | 52 | 54 | 5 | 39 |

LSD_{0.05} for isolate: 4.

LSD_{0.05} for interaction: 6.

The growth of *T. aggressivum* f. *europaeum* isolates on the standard agar medium was considerably weaker than on the manure one. In addition, in this case, significant differences in culture growth of the examined isolates were recorded. The best growth was observed in the case of T75/8 and T90/5 isolates (31 mm), while the worst – in the case of T17/3 and T30/B (23 mm; Table 2). Isolates deriving from West European mushroom-growing houses exhibited similar growth on the discussed medium.

The applied media highly differentiated the growth of examined isolates. For example, isolate T30/B which grew very poorly on the standard agar medium exhibited good growth on the manure medium. Similarly, the T17/3 isolate which grew badly on the standard agar medium turned out to grow well on the manure medium. Analyzing culture growth of the isolates on the standard agar medium, it

Table 2

Colony diameter of *Trichoderma aggressivum f. europaeum* isolates after three days of growth on standard agar medium (mm)

| Isolate | Temperature (°C) | | | | | Mean |
|------------|------------------|----|----|----|----|------|
| | 15 | 20 | 25 | 30 | 35 | |
| CPK 361 | 11 | 25 | 43 | 46 | 10 | 27 |
| CBS 100526 | 14 | 22 | 40 | 43 | 12 | 26 |
| T7/23 | 9 | 24 | 41 | 45 | 8 | 25 |
| T9/25 | 12 | 27 | 44 | 44 | 9 | 27 |
| TM/11 | 10 | 20 | 39 | 41 | 8 | 24 |
| TS/13 | 11 | 24 | 41 | 44 | 11 | 26 |
| TA/12 | 15 | 27 | 45 | 48 | 12 | 29 |
| TR/7 | 9 | 23 | 47 | 49 | 13 | 28 |
| T12/4 | 8 | 24 | 44 | 47 | 9 | 26 |
| T17/3 | 8 | 22 | 36 | 38 | 10 | 23 |
| TX/32 | 9 | 23 | 37 | 43 | 12 | 25 |
| T31/1 | 14 | 26 | 46 | 50 | 14 | 30 |
| T40/5 | 10 | 25 | 48 | 50 | 12 | 29 |
| T17/S | 6 | 23 | 40 | 41 | 9 | 24 |
| T24/A | 12 | 31 | 46 | 48 | 10 | 29 |
| T30/B | 7 | 20 | 38 | 43 | 9 | 23 |
| T11/8 | 8 | 26 | 48 | 52 | 14 | 30 |
| T75/8 | 13 | 27 | 49 | 49 | 15 | 31 |
| T81/2 | 7 | 22 | 38 | 40 | 9 | 23 |
| T90/5 | 15 | 28 | 47 | 53 | 12 | 31 |
| T93/7 | 10 | 24 | 45 | 49 | 8 | 27 |
| T97/2 | 13 | 23 | 41 | 48 | 9 | 27 |

LSD_{0.05} for isolate: 4.

LSD_{0.05} for interaction: 6.

was found that as in the case of growth on the manure medium, the best growth was recorded at the temperatures of 25 and 30°C (Fig. 2). The examined cultures grew much worse at the temperature of 20°C, whereas at the temperatures of 15°C and 35°C its growth was very weak.

Discussion

The obtained results are in agreement with those reported by Samuels et al. (2002). The above-mentioned researchers studied growth of various isolates of the *Trichoderma* genus growing on PDA and SNA media. Mycelium growth of the

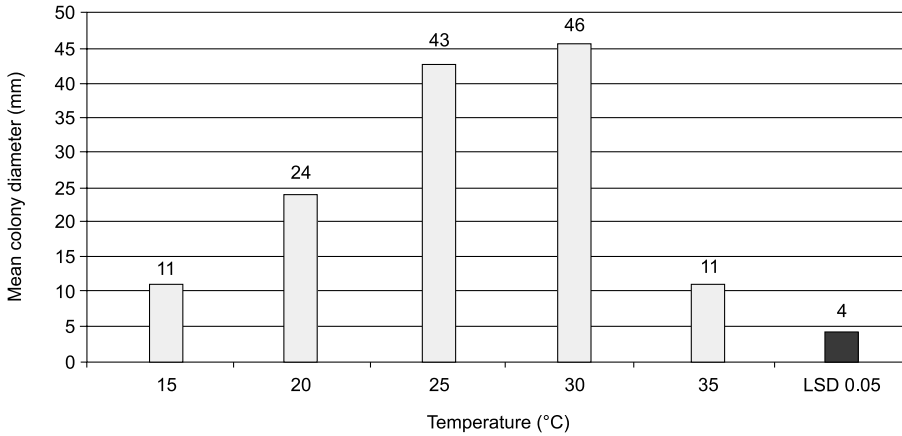


Fig. 2. Mean colony diameter of 22 *Trichoderma aggressivum f. europaeum* isolates after three days of growth on standard agar medium in relation to temperature (mm)

T. aggressivum f. europaeum species on the PDA medium was, in general, fairly similar to the growth observed here on the manure medium. On the other hand, the mycelium growth of the examined *T. aggressivum f. europaeum* isolates on the standard agar medium was weaker than on the SNA medium used by Samuels et al. (2002). Moreover, the obtained results also fully corroborate the conclusion of the above-mentioned researchers that the aggressive isolates of *T. aggressivum f. europaeum* exhibit very poor growth at the temperature of 35°C. The mycelium of tested isolates grew the fastest at the temperatures of 25 and 30°C. It suggests that it is very difficult to control green moulds in mushroom cultivation as the optimal growth of mushroom mycelium is also observed at the temperature of 25°C. Investigations carried out by the authors also confirmed that aggressive isolates of *T. aggressivum f. europaeum* exhibit different growth on different media. Similar results were obtained by Szczech et al. (2008) in experiments conducted in Poland.

Conclusions

1. The mycelium growth of *T. aggressivum f. europaeum* isolates depended on type of agar medium. The mycelium growth rate was higher on the manure medium than on the standard agar medium.

2. The examined *T. aggressivum f. europaeum* isolates showed considerable differences in growth depending on temperature. Temperatures 25 and 30°C turned out to be optimal for all the examined isolates on both media. Mycelium growth underwent rapid decrease at 35°C.

Streszczenie

TEMPERATURA I RODZAJ POŻYWKI JAKO CZYNNIKI RÓŻNICUJĄCE WZROST AGRESYWNYCH IZOLATÓW *TRICHODERMA AGGRESSIVUM* F. *EUROPAEUM*

Celem przeprowadzonych badań było określenie wpływu temperatury oraz rodzaju pożywki na wzrost grzybni agresywnych izolatów *Trichoderma aggressivum* f. *europaeum*. W doświadczeniu użyto dwóch izolatów *T. aggressivum* f. *europaeum* pochodzących z pieczarkarni Europy Zachodniej – CPK 361 z Austrii i CBS 100526 z Holandii oraz 20 izolatów pochodzących z polskich pieczarkarni i boczniakarni. Wzrost grzybni badano w temperaturach: 15, 20, 25, 30 i 35°C na dwóch pożywkach agarowych: standardowej mineralnej oraz organicznej obornikowej. Grzybnia badanych izolatów wykazała na obu pożywkach zróżnicowany wzrost. Na pożywce obornikowej uzyskano lepszy średnio wzrost grzybni niż na pożywce standardowej. Izolaty *T. aggressivum* f. *europaeum* wykazały duże zróżnicowanie wzrostu w zależności od temperatury. Temperatury 25 i 30°C były optymalne dla wzrostu wszystkich badanych izolatów.

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