Scientific Bulletin. Series F. Biotechnologies, Vol. XIX, 2015
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

INFLUENCE OF DIFFERENT TEMPERATURES AND RELATIVE HUMIDITIES ON *IN VITRO* GERMINATION OF THREE ENTOMOPATHOGENIC FUNGAL STRAINS OF *BEAUVERIA BRONGNIARTII*

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Abstract

The entomopathogenic fungus Beauveria brongniartii Sacc. (Petch) *is the most important natural enemy of Melolontha melolontha L. Three strains of B. brongniartii isolated from natural diseased M. melolontha larvae were tested in order to assess the effects of temperature and relative humidity on conidial germination, the most important factors during the initialization of infection process. Conidial germination was examined at temperatures ranging from 4° to 33°C, on PDA medium. The effect of relative humidity was tested using the method described by Beyer et al. (2004) with slight changes. RHs tested were 29.8% $CaCl_2$, 52.6% $Ca(NO_3)_2$, 75.3% NaCl,, 84.7% KCl,, 92.7% KNO_3 and 100% deionized water. The results show that no strain germinated at 4 and 10°C. At 22°C all isolates exceeded 95% germination after 24 h. Temperatures over 25°C had a negative influence on the rate of spore germination. Incubation of conidia at low humidity (RH 29%) suppressed germination for all strains. All relative humidities over 29.8% were found favorable for germination of all this strains.*

Key words: *Beauveria brongniartii, temperature, humidity, germination.*

INTRODUCTION

The European cockchafer larvae (chafer grubs) cause serious damage in Romanian forest nurseries. The entomopathogenic fungus *Beauveria brongniartii* Sacc. (Petch) is the most important natural enemy of *Melolontha melolontha*. *Beauveria* species attack their host insect generally percutaneously. Temperature can affect an entomopathogen in different ways by influencing the germination, growth and viability of the fungus on and in the host insect and in the environment. Humidity is a very important environmental factor affecting the efficacy and survival of entomopathogens (Zimmerman, 2007). The importance of temperature and humidity on infection process was demonstrated by studies since 1965 (Clerk and Madelin), then the research carried out by Benz (1987), Daoust and Pereira (1986) proved that the temperature and the humidity are the most important factors that affect the conidial germination rates. James et al. (1998) consider that the effect of humidity is the greatest during the initiation of infection, because it is primarily a requirement for conidial germination.

A review recently published (Jaronski, 2009) also emphasizes the importance of temperature and humidity, as abiotic factors affecting the fungal efficacy in foliar and soil applications. In vitro results on conidial germination are presented by Lazzarini et al. (2006).

In this paper are presented the results of laboratory tests performed with three indigenous *B. brongniartii* isolates, in order to assess the effects of temperature and relative humidity on *in vitro* conidial germination.

MATERIALS AND METHODS

Source of conidia

Three isolates of *B. brongniartii* (ICDPP#2-4, re-named here 1Bbg, 2Bbg, 3Bbg respectively) used in this study were from Culture

Collection of Entomopathogenic Fungi maintained in the Department of Useful Organisms, (Research - Development Institute for Plant Protection, Bucharest). The identity of the isolates as *B. brongniartii* was confirmed by polymerase chain reaction (PCR) (http://www.icdpp.ro, 2010). The strains were initially isolated from natural diseased *Melolontha melolontha* grubs.

B. brongniartii conidia were obtained from four months old sporulating cultures grown at 25°C on PDA in tubes. Conidia were harvested from the surface of these cultures by washing, using sterile distilled water with 0.1% Tween® 80. Conidial concentrations were determinates by counting in haemocytometer.

Germination at various temperatures

Conidial germination was examined at temperatures of 4°, 10°, 15.5°, 20°, 22°, 25°, 28°, 30°, 33°C on PDA medium. Spore suspension was calibrated at a concentration of 10^7 conidia/ml diluted in sterile distilled water to which was added Tween 80 (0.01%). Petri dishes (96 mm) were inoculated with 0.5 ml of conidial suspensions for each isolate, spread with a sterile Drigalski spatula and incubated under saturated atmosphere in total darkness. Petri dishes were examined after 8, 16, 24, 32 h post-inoculation using a microscope under 400 × magnifications. Germination process was stopped by dripping a lacto phenol cotton-blue solution. Conidia were considered germinated when germinative tube length exceeded 1 to 1.5 times their length. For each plate, four areas of at least one hundred conidia were counted.

Germination at various relative humidities

The effect of relative humidity was tested using the method described by Beyer et al. (2004) with slight changes. Briefly, the inner side of Petri dish lids was coated with PDA and inoculated as was described before. After droplet drying (1 hour), the dishes were filled with constant humidity solutions (Wexler, 1995) or deionized water and incubated for 24 h at 25° C. Subsequently, the dishes were closed and sealed with parafilm, such that the spores attached to the lid were incubated above constant humidity solutions or pure water. RHs tested were 29.8% /$CaCl_2$, 52.6% /$Ca(NO_3)_2$, 75.3% /NaCl, 84.7% /KCl, 92.7% /KNO_3 and 100% /deionized water.

Data were analysed using the Kuskal-Wallis nonparametric test of the AnalistSoft Inc., BioStat v2009.

RESULTS AND DISCUSSIONS

Influence of temperature on conidial germination

The temperature parameter influences the germination of tested strains. The period of time required until over 95% of spore to germinate was 24 h for all strains at 22°C (Table 1, 2, 3).

No strain germinated at 4 and 10°C even after 32 h, germination started from 15.5°C. At this temperature all isolates germinated after 24 h post–inoculation, 2Bbg strain being distinguishable by the highest percentage of germination, both after 24 and after 32 h (Table 2). At 20°C the germination started after 16 h just in case of 2Bbg strain, reaching the maximum rate after 32 h.

At 22°C all isolates exceeded 95% germination after 24 h, strain 2Bbg standing out at a rate over 98%. The speed of germination is at a higher percentage at 22°C than at 25°C for each isolate. No germination was observed after an incubation of 8 h at all temperatures.

Table 1. Effect of temperature on conidial germination of 1Bbg strain under saturated atmosphere

Temperature (°C)	Germination rates after specified number of hours (means+SD) (%)			
	8	16	24	32
4	0	0	0	0
10	0	0	0	0
15.5	0	0	2.3±0.5	21.6±1.2
20	0	0	9.7±0.4	†
22	0	3.0±0.4	94.5±0.3	†
25	0	2.5±0.2	95±0.7	†
28	0	0	0	0
30	0	0	0.9±0.5	0
33	0	0	0	0

† Germination rates over 99%

Table 2. Effect of temperature on conidial germination of 2Bbg strain under saturated atmosphere

Temperature (°C)	Germination rates after specified number of hours (means+SD) (%)			
	8	16	24	32
4	0	0	0	0
10	0	0	0	0
15.5	0	0	14.6±0.5	67.5±0.8
20	0	4.5±0.2	53.4±0.7	†
22	0	22.9±0.5	98.0±0.2	†
25	0	20.5±0.7	†	†
28	0	0	0	0
30	0	1.3±0.7	1.76±0.8	0.2±0.3
33	0	0.9±0.9	0.17±0.2	0.1± 0.9

† Germination rates over 99%

Table 3. Effect of temperature on conidial germination of 3Bbg strain under saturated atmosphere

Temperature (°C)	Germination rates after specified number of hours (means+SD) (%)			
	8	16	24	32
4	0	0	0	0
10	0	0	0	0
15.5	0	0	2.7±0.4	48.8±0.4
20	0	0	21.9±0.4	†
22	0	10.0±1.2	96.5±0.9	†
25	0	5.6±0.3	95.6±0.3	†
28	0	0	0	0
30	0	0	0	0
33	0	0	0	0

† Germination rates over 99%

Temperatures over 25°C had a negative influence on the rate of spore germination, decreasing under 1.7% for 2Bbg strain (Table 2) and being almost not observed for 1Bbg and 3Bbg strains.

Kruskal-Wallis ANOVA showed no significant differences between strains regarding the influence of temperature, with all this, the strain 2Bbg has a faster speed of germination than the two other strains, at all temperatures.

The optimum temperature for germination was 22°C for all this isolates, in accordance with literature findings (Zimmerman, 2007). Although no germination occurred at 4 and 8°C after 32 h, during four months of preservation at 4±1°C, vegetative development was observed only for 2Bbg strain. Müller-Kögler (1960) found that the conidia of *B. brongniartii* (syn. *B. tenella*) have somewhat higher degree of germination than those of *B. bassiana* after three or four months on artificial media (Steinhaus, 1964).

Effect of relative humidity on conidial germination

Incubation of conidia at low humidity (RH 29%) suppressed germination for all strains (Table 4). All relative humidities over 29.8% were found favorable for germination of all this isolates. Over this percent, there is no variability between the relative humidity and percent of germination. The highest rate of germination was registered at 92.7 and 100% RH for 2Bbg and 3Bbg strains.

Table 4. Effect of relative humidity on conidial germination of *B. brongniartii* isolates after 24 hours of incubation at 25° C

Relative Humidity (%)	Germination rates after 24 hours (means+SD) (%)		
	1Bbg	2Bbg	3Bbg
29.8	0	0	0
52.6	76.2±1.3	92.13±1.5	94.3±2.3
75.3	96.9±2.3	98.5±2.2	96.7±1.7
84.7	92.2±1.8	62.7±3.4	93.6±1.6
92.7	96.1±0.8	†	†
100	95.8±3.4	†	†

† Germination rates over 99%

Our results showed that RH between 52.6 and 100% is not a limiting factor for germination of *B. brongniartii* isolates, which is in contrast with findings of Luz and Fargues (1997) on humidity requirements over 90% for *B. bassiana*. Also Gillespies and Crawford (1986) noted that for optimal development of most entomopathogenic hyphomycetes, the relative humidity should be above 97%. The present study is in accordance to Piatti et al. (1995) who observed that humidity requirement for fungal growth of *B. brongniartii* in soil was 57%. Also Padmini and Padmaja (2010) reported that vegetative growth and sporulation were excellent at 60% RH and 30°C temperature for twenty isolates of *Beauveria* species. Ferron (1977) found that the infection and incubation phases are not affected by relative humidity.

CONCLUSIONS

Our studies on temperature and humidity requirements for conidial germination of three *B. brongniartii* Romanian isolates, pathogenic for *Melolontha melolontha* revealed that the optimum temperature for conidial germination is 22°C; the relative humidity between 52.6 and 100% does not influence negatively the *in vitro* germination of this strains.

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