Brazilian Journal of Microbiology 46, 1, 67-74 (2015)
ISSN 1678-4405
DOI: http://dx.doi.org/10.1590/S1517-838246120131077

Research Paper

Effect of surfactants and temperature on germination and vegetative growth of *Beauveria bassiana*

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Submitted: October 9, 2013; Approved: June 6, 2014.

Abstract

Three non-ionic surfactants: Tween20, Tween80 and Breakthru® were screened for their effects on spore germination and mycelial growth rates and for their influence on three isolates of *Beauveria bassiana* spore germination at various temperatures. Tween20 and Tween80 were compatible with all the *B. bassiana* isolates in the germination studies, but inhibited germination at higher surfactant concentrations, irrespective of the conidial concentrations. Breakthru® had an inhibitory effect on germination even at the lowest concentration of 0.1% on all the *B. bassiana* isolates. The effects of the surfactants on spore germination did not correspond with their effects on colony growth. Conidial viability within the same formulation declined significantly with increases in temperature, irrespective of the surfactant. The optimal temperature for conidial germination of *B. bassiana* isolates was approximately 25 °C with an upper limit at 30 °C. Isolate 7320 was identified as the least affected by the different surfactants. This isolate was able to germinate rapidly in a broad temperature range of 25-30 °C after 24 h, this characteristic being an essential factor in controlling house fly populations in poultry houses.

Key words: *Beauveria bassiana*, surfactant, temperature, conidia germination, mycelial growth.

Introduction

House flies (*Musca domestica* L.) are major pests of poultry production systems in tropical and subtropical countries. Up to now, control strategies have been dependent on the use of synthetic chemical insecticides. However, recognition of associated problems such as non-target effects, environmental pollution as well as the high economic costs involved, have prompted the development of alternative control strategies. *Beauveria bassiana* (Balsama) Vuillemin is one of the most ubiquitous and extensively studied entomopathogenic fungi (Feng *et al.*, 1994; Hajek and St. Leger, 1994). This entomopathogenic fungus has also been intensively studied with the aim of development of commercial mycopesticides for the management of insect pests (Burges, 1998; Butt *et al.*, 2001).

In our previous investigations (Mwamburi *et al.*, 2010), a number of *B. bassiana* isolates that showed high virulence to adult house flies were identified, but a range of factors need to be considered before selecting the isolates for further use. Prolonged conidial survival in the field would help to maximize mortality of target insects. However, prolonged exposure to high temperatures limits the survival of entomopathogenic fungi in the field (Benz, 1987; Carruthers *et al.*, 1985; Ekesi *et al.*, 1999; Fargues *et al.*, 1992; Ferron *et al.*, 1991; Roberts and Campbell, 1977; Vestergaard *et al.*, 1995). The thermal constraints are not only as a result of ambient conditions, but also those achieved through host thermoregulation. For instance, some insects elevate their body temperature through basking in the sun (Chappell and Whitman, 1990) and such activity has been shown to reduce disease incidence of *Entomophthora muscae* (Cohn) Fres. in house flies (Wat-

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son *et al.*, 1993), *Entomophaga grylli* (Fres.) Batko (Carruthers *et al.*, 1992), *B. bassiana* (Bals.) Vuill. (Inglis *et al.*, 1996) and *Metarhizium flavoviride* Gams and Rozsypal (Fargues *et al.*, 1997; Inglis *et al.*, 1996) in acridids.

The surfactant used is also recognized as a critical component in assisting conidia of a pathogen to germinate and infect the target organism. Surfactants can have a range of effects on fungal spore germination and mycelial growth. Therefore there is need for careful evaluation for compatibility of surfactants with conidia prior to their use in formulations (Daoust, 1983). The selection of an surfactant can enhance the uptake and transport of fungal spores and therefore play an important role in increasing the efficacy of a biocontrol agent such as *Beauveria*. This aim of this study was to evaluate the effects of various surfactants on the germination and tolerance of *Beauveria bassiana* isolates (7320, 7569 and 7771) spores formulated at different conidial densities at three different temperatures regimes.

Materials and Methods

Fungal isolates

Three isolates of *B. bassiana* (Isolates 7320, 7569, 7771) were used in this study. These isolates were originally obtained from the Plant Protection Research Institute (Agricultural Research Council, 1134 Park Street, P.O. Box 8783 Hatfield, Pretoria 0001), Pretoria.

Fungal cultures

The fungi were grown on Sabourand Dextrose Agar (SDA) in Petri dishes and incubated for 10 d for fungal growth and conidial production. For viability tests, conidia were removed using a brush, suspended in distilled water and different surfactants, and vortexed for 2 min to produce a homogenous suspension. Conidia were mixed with surfactants prior to addition of water to obtain homogenous suspensions. The stock formulation of each concentration was filtered using a sterile muslin cloth. All conidial formulations had the dose adjusted to 10^4, 10^5, 10^6, 10^7 and 10^8 conidia mL^{-1} using a Neubauer's chamber.

Surfactants

Three surfactants (Tween20, Tween80 and Breakthru® (Evonik Degussa Africa (Pty) Ltd. Co. New Rd, Halfway House 1682, South Africa)) were used to measure conidial germination and mycelial growth. Water was used as a control.

Effects of different surfactants on Beauveria bassiana conidial viability

A factorial design was set up consisting of three fungal isolates (Isolates 7320, 7569, and 7771), four conidial densities (10^5, 10^6, 10^7 and 10^8 conidia mL^{-1}), three surfactants (Tween20, Tween80, Breakthru®) and water as a control, with five surfactant concentrations (0, 0.1, 0.5, 1 and 5% v/v or w/v). The effects of surfactants on conidial germination was evaluated by incorporating surfactants directly into 1.5% water agar at 0, 0.1, 0.5, 1 and 5% (v/v or w/v). Agar discs (16 mm diameter) were then cut with a cork borer and placed onto supporting slides. Drops (10 µL) of each conidial suspension were placed on the discs and spread evenly on the surface. The glass slides supporting the agar discs were placed in Petri dishes and incubated at approximately 21 °C. After 24 h of incubation, 12.5 µL of lactophenol-cotton blue were placed on the agar discs to arrest germination of conidia. A conidium was considered to have germinated when the length of the germtube was greater than its width, or when a sessile appressorium was produced. Several randomly selected fields of view were examined using a compound microscope until a total of 300 conidia per replicate had been assessed.

Effects of different surfactants on Beauveria bassiana mycelial growth

To measure mycelial growth, Petri dishes containing 20 mL PDA were amended with the same surfactant concentrations. Each dish was inoculated with a 6 mm diameter mycelial-agar plug obtained from the margin of a 7-day old culture. Dishes of each treatment were incubated for 10 d at constant dark at 21 ± 1 °C and were removed at 48 h intervals for assessment of mycelial growth. Colony growth was recorded as mean perpendicular radius minus the diameter of the inoculum plug (6 mm).

Effect of temperature on conidial germination

To investigate the effect of temperature on conidial viability, a conidial suspension (1 mL) of each isolate (10^8 conidia mL^{-1}) was mixed with the surfactant and plated onto Petri dishes containing 20 mL PDA that were amended with the same surfactant. Plates were incubated in the dark at 25, 30 and 35 ± 1 °C. Conidial viability tests were carried out with readings after 24 h and 48 h of incubation at 21 °C to allow time for conidia recovery from any adverse effects caused by temperature. Conidia were observed at 400x magnification and germination was recorded when the germ tube was visible. A minimum of 300 conidia per plate were evaluated.

Statistical analysis

The viability experiments had factorial designs with four factors. Analysis of variance (ANOVA) on conidial viability data was performed using GENSTAT, after transforming the percent germination data to Arcsine $\sqrt{}$ (%/100) for normal distribution and homogeneity of variances (Sokal and Rohlf, 2012). This data is presented in the tables together with the untransformed means and were used in statistical analysis. Means were compared using Least Significant Difference (LSD). The results are presented both as untransformed and transformed data.

The mycelial growth rate (K_r) was calculated in millimetres per 24 h using simple linear regression and was used as the main parameter to evaluate the influence of temperature on fungal growth (Fargues *et al.*, 1992). ANOVA was performed on the growth rates and means were compared using LSD.

Results

Effects of different surfactants on Beauveria bassiana conidial viability

Conidial concentration, surfactants, surfactant concentration and the interaction of these factors affected germination of conidia ($p < 0.001$) of isolates of *B. bassiana.* The germination of conidia of three *B. bassiana* isolates in response to the three surfactants (Tween20, Tween80 and Breakthru®), surfactant concentration and conidial densities are shown in Figure 1. The surfactants generally inhibited germination at higher surfactant concentrations and at all conidial concentrations for all the three isolates of *Beauveria* (Figure 1). Tween20 showed varied effects on the three *Beauveria* isolates. For Isolate 7320, conidial germination remained fairly constant initially, declining by at least 30% as the concentration of Tween20 increased up to 1%, after which there was a more obvious decrease and germination dropped to 66, 56, 49 and 42% in 10^5, 10^6, 10^7 and 10^8 conidia mL^{-1} concentrations respectively (Figure 1).

For Isolate 7569, the addition of Tween20 did not stimulate conidial germination, even at low concentrations of 0.1%. Instead, the addition of Tween20 resulted in an immediate rapid decline in germination, *e.g.*, at low conidial concentrations of 10^5, 10^6 and 10^7 conidia mL^{-1}, germination was reduced by 40-50%, while at a higher conidial concentration of 10^8, germination was reduced by approximately 20% with a concentration of 0.1% Tween20. Increases of Tween20 concentrations beyond 0.1% resulted in a gradual decline in germination (Figure 1). In the case of Isolate 7771, Tween20 stimulated conidial germination at 0.1% concentration, but inhibited conidial germination at higher concentrations (Figure 1).

Tween80 inhibited germination of Isolate 7320 at 0.1% concentration, but stimulated germination at 0.5 and 1.0% concentrations. For example, at conidial concentrations of 10^6, 10^7 and 10^8, Tween80 had a stimulatory effect on germination, increasing by approximately 10% for 10^7 and 10^8 and 30% for 10^6 conidial concentrations. The same surfactant concentrations had no effect on the 10^5 conidial concentrations. Conidial germination dropped sharply with a 1.0% Tween80 concentration, for all concentrations of conidia. Addition of 0.1% Tween80 had a slight stimulatory effect on germination of Isolate 7569 conidia, but concentrations between 0.1-0.5% had substantial inhibitory effects on germination. Tween80 concentrations higher than 0.5% had slight (5-12%) stimulatory effects on germination. For the Isolate 7771, 0.1% concentration of Tween80 stimulated germination; however, surfactant concentrations higher than 0.1% had inhibitory effects on conidial germination (Figure 1).

Breakthru® had an inhibitory effect on all the *B. bassiana* isolates at all concentrations. For Isolate 7320, although the 0.1% concentration caused a slight inhibition of germination, a significant decline of conidial germination was observed as Breakthru® concentration was increased, resulting in approximately 70% inhibition of germination with the 5% Breakthru® concentration. Although addition of Breakthru® inhibited conidial germination of Isolates 7569 and 7771, the trends differed from that observed with 7320. For both Isolates 7569 and 7771, addition of Breakthru® at a low concentration of 0.1% resulted

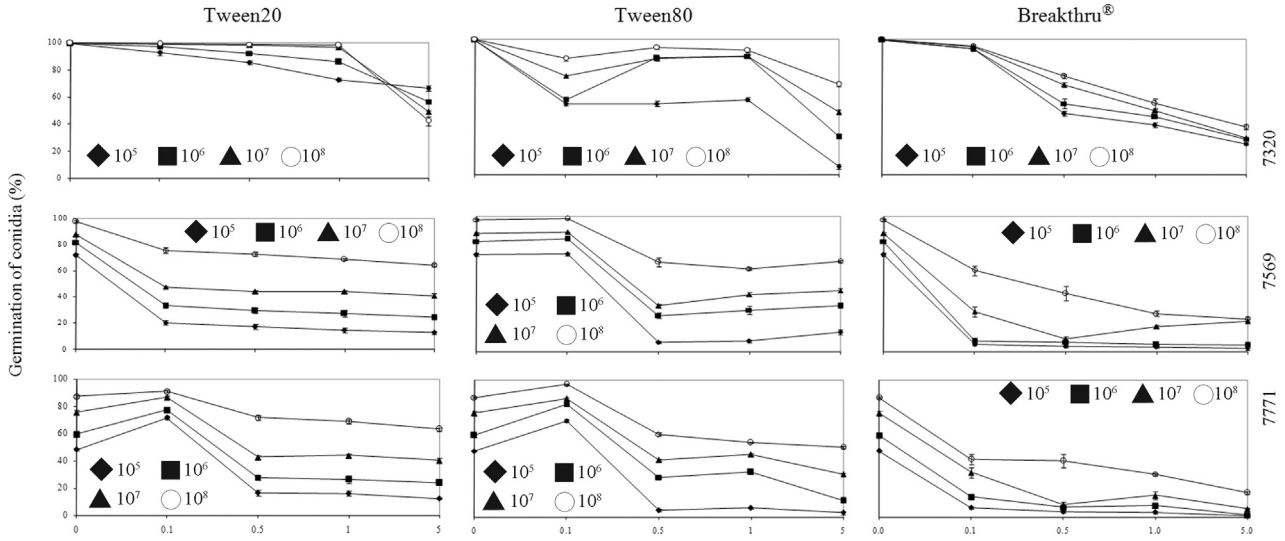

Figure 1 - Effects of Tween20, Tween80 and Breakthru® at various concentrations (0, 0.1, 0.5, 1 and 5%) on the germination of conidia of three isolates of *Beauveria bassiana* at four conidial densities.

in an immediate rapid drop of > 40% of conidial germination (Figure 1).

There were significant differences in germination among concentrations of the similar surfactants within the same conidial concentration for all the three isolates ($p < 0.001$).

Effects of different surfactants on Beauveria bassiana mycelial growth rate

Isolates, surfactants and surfactant concentration and their interactions except isolate x surfactant x surfactant concentration had significant effects ($p < 0.05$) on radial mycelial growth (K_r) of isolates of *B. bassiana*. Tween20 caused a non-significant mycelial growth reduction at 0.1% concentration in 7320 and a significant inhibition after 0.1% concentration that remained fairly constant at higher concentrations (Table 1). While an increase in concentration of Tween20 presented significant reductions in Isolate 7569, fewer differences were observed in Isolate 7771 (Table 1).

The effect of Tween80 on the K_r of the *B. bassiana* isolates was similar to that of conidial germination. The growth of Isolate 7320 was inhibited by 0.1% concentration of Tween80, stimulated at 0.5 and 1.0% concentrations and inhibited at concentrations higher than 1.0%. Tween80 inhibited growth of Isolate 7569 at 0.1% concentration and these were non-significant reductions at higher concentrations. Growth of Isolate 7771 was not affected by Tween80 concentrations of up to 1% (Table 1).

For all the *B. bassiana* isolates, moderate reductions in mycelial growths were observed as a result of increasing concentrations of Breakthru®. However, fewer differences were observed for Isolate 7771 compared to Isolates 7320 and 7569 (Table 1).

Effect of temperature of germination

The effects of surfactant, temperature, incubation time and the interaction of these factors significantly affected ($p < 0.001$) the conidial germination of the various isolates of *B. bassiana*. Comparisons of the mean conidial germination levels between at each temperature within

Table 1 - Effects of five concentrations of three surfactants (Tween20, Tween80 and Breakthru®) on the radial growth rate ($K_r \pm$ se) of three isolates (7320, 7569 and 7771) of *Beauveria bassiana.*

Surfactant	Surfactant concentration (%)	Radial mycelial growth rate (K_r in mm day $^{-1}$) ± se		
		Isolates		
		7320	7569	7771
Tween20	0	1.08 ± 0.08 ^{f}	1.46 ± 0.06 ^{f}	1.13 ± 0.08 ^{b}
	0.1	1.02 ± 0.07 ^{def}	1.17 ± 0.1 ^{de}	1.54 ± 0.3 ^{c}
	0.5	0.8 ± 0.09 ^{ab}	1.22 ± 0.17 ^{e}	1.11 ± 0.09 ^{b}
	1	0.9 ± 0.09 ^{bcd}	1.08 ± 0.13 ^{cd}	1.13 ± 0.09 ^{b}
	5	0.8 ± 0.09 ^{ab}	0.88 ± 0.09 ^{b}	1.10 ± 0.11 ^{b}
Tween80	0	1.08 ± 0.08^{f}	1.46 ± 0.06 ^{f}	1.13 ± 0.08 ^{b}
	0.1	0.96 ± 0.1^{cdef}	1.07 ± 0.13 ^{c}	1.07 ± 0.09 ^{ab}
	0.5	1.04 ± 0.12^{def}	1.12 ± 0.07^{cd}	1.05 ± 0.07 ^{ab}
	1	1.06 ± 0.12^{ef}	1.13 ± 0.1 ^{cd}	1.11 ± 0.09 ^{a}
	5	0.87 ± 0.1^{bc}	1.12 ± 0.13 ^{cd}	0.86 ± 0.15 ^{ab}
Breakthru®	0	1.08 ± 0.08^{f}	1.46 ± 0.06 ^{f}	1.13 ± 0.08 ^{b}
	0.1	0.96 ± 0.12^{cdef}	1.26 ± 0.14 ^{e}	1.01 ± 0.09 ^{ab}
	0.5	1.04 ± 0.13^{def}	1.11 ± 0.13 ^{cd}	0.86 ± 0.1^{ab}
	1	0.92 ± 0.14^{bcde}	1.13 ± 0.13 ^{cd}	0.84 ± 0.08 ^{ab}
	5	0.69 ± 0.09^{a}	0.72 ± 0.1 ^{a}	0.68 ± 0.06 ^{a}
F-ratio		55.28	67.15	34.23
p-value		< 0.001	< 0.001	< 0.001
LSD		0.07	0.053	0.06
%CV		11.5	7	6.5
Effect		**	**	**

Means followed by the same letter within the same column are not significantly different ($p > 0.05$); F and p values after square root-arcsine transformation; ** Significant at $p \leq 0.001$.

each surfactant and between the isolates within each exposure time, 24 and 48 h after incubation are shown in Table 2. Factorial analysis of variance revealed more significant differences among surfactants at 24 h than 48 h. Conidial viability within the same formulation declined significantly with increases in temperature, irrespective of formulation. This effect was more pronounced for Isolate 7771 and less prominent for Isolate 7320 (Table 2).

All the isolates showed more than 90% germination after 48 h at all temperatures. At a temperature of 30 ± 1 °C, < 40% conidial germination was observed in all surfactants for the tested isolates, except Isolate 7320. With this isolate > 75% germination was observed at 30 °C with all the surfactants (Table 2). No delay in germination was observed at this temperature compared to germination at 25 °C. At a temperature of 35 °C, all the isolates showed a significant delay or decrease in relative percentage germination after 24 h but reached > 90% after 48 h. At a temperature of 35 °C, all *B. bassiana* isolates failed to germinate within 24 h. Only Isolate 7320 germinated, but the levels of germination were 0.18, 0.04 and 0.04% in water, Tween20 and Tween80, respectively.

The effect of water on germination can be separated into different responses with increasing temperature. While high germination levels were obtained with Isolate 7320 at 25 °C, Isolates 7569 and 7771 exhibited moderate germina-

Table 2 - Conidial viability (% ± se) of three isolates of *Beauveria bassiana* in different surfactants, 24 h and 48 h after incubation at three temperatures (25, 30 and 35 °C).

		Mean germination of conidia (%)					
		Isolates					
		7320		7569		7771	
Surfactant	Temperature	24 h	48 h	24 h	48 h	24 h	48 h
Water	25	98.4 ± 0.51 (1.46)^{e}	99.2 ± 0.37 (1.50)^{a}	61.0 ± 3.32 (0.90)^{e}	98.6 ± 0.51 (1.47)^{a}	63.8 ± 3.46 (0.93)^{g}	98.6 ± 0.51 (1.47)^{a}
	30	77.2 ± 0.86 (1.07)^{d}	98.4 ± 0.51 (1.46)^{a}	0.80 ± 0.12 (0.09)^{d}	98 ± 0.32 (1.43)^{a}	48.4 ± 1.08 (0.77)^{d}	98.4 ± 0.51 (1.46)^{a}
	35	0.18 ± 0.09 (0.04)^{a}	98.4 ± 0.40 (1.46)^{a}	0 ± 0 (0)^{a}	95.4 ± 1.44 (1.36)^{a}	0 ± 0 (0)^{a}	98.4 ± 0.51 (1.46)^{a}
Tween20	25	98.4 ± 0.51 (1.46)^{e}	98.6 ± 0.40 (1.47)^{a}	34.6 ± 1.86 (0.63)^{d}	98 ± 0.32 (1.43)^{a}	57.2 ± 3.65 (0.86)^{i}	96.8 ± 1.77 (1.42)^{a}
	30	86.8 ± 1.07 (1.20)^{cd}	98.2 ± 0.37 (1.44)^{a}	27.8 ± 1.39(0.55)^{de}	97.4 ± 0.68 (1.41)^{a}	38.6 ± 2.73 (0.67)^{de}	95.6 ± 1.5 (1.37)^{a}
	35	0.04 ± 0.02 (0.01)^{a}	98.6 ± 0.40 (1.47)^{a}	0 ± 0 (0)^{a}	95.2 ± 1.39 (1.36)^{a}	0 ± 0 (0)^{a}	92.4 ± 2.18 (1.30)^{a}
Tween80	25	98.0 ± 0.95 (1.46)^{e}	98.8 ± 0.37 (1.47)^{a}	29.4 ± 3.14 (0.57)^{f}	97.8 ± 0.37 (1.42)^{a}	75.2 ± 1.59 (1.05)^{h}	98 ± 0.55 (1.44)^{a}
	30	83.0 ± 3.30 (1.15)^{bc}	98.2 ± 0.20 (1.44)^{a}	31.2 ± 1.85 (0.59)^{b}	95.2 ± 1.39 (1.36)^{a}	40 ± 3.54 (0.68)^{f}	98.8 ± 0.37 (1.47)^{a}
	35	0.04 ± 0.02 (0.01)^{ab}	98.2 ± 0.37 (1.44)^{a}	0 ± 0 (0)^{a}	95 ± 1.3 (1.35)^{a}	0 ± 0 (0)^{a}	98.8 ± 0.37 (1.47)^{a}
Breakthru®	25	97.2 ± 0.86 (1.42)^{e}	98.6 ± 0.60 (1.48)^{a}	29.8 ± 1.77(0.58)^{de}	97.0 ± 0.32 (1.4)^{a}	21.6 ± 2.09 (0.48)^{c}	95.8 ± 1.59 (1.38)^{a}
	30	87.4 ± 2.50 (1.22)^{bc}	98.4 ± 0.51 (1.46)^{a}	7.0 ± 0.95 (0.27)^{c}	95.0 ± 1.38 (1.35)^{a}	15 ± 1.92 (0.39)^{b}	98.2 ± 0.37 (1.44)^{a}
	35	0 ± 0 (0)^{ab}	98.0 ± 0.32 (1.43)^{a}	0 ± 0 (0)^{a}	93.8 ± 1.02 (1.32)^{a}	0 ± 0 (0)^{a}	97.2 ± 0.73 (1.41)^{a}
F-ratio		2.52	0.29	104.68	0.41	30.15	1.35
p-value		0.034	0.941	< 0.001	0.868	< 0.001	0.255
LSD		0.081	0.072	0.053	0.068	0.067	0.093
%CV		7.2	3.9	12	3.8	10	5.1
Effect		*	NS	**	NS	**	NS

Values in parenthesis are square-root arcsine transformed.
Means followed by the same small letter within the same column are not significantly different at $p < 0.05$.
*Significant at $p < 0.05$; **Significant at $p \leq 0.001$; NS - Not significant.

tion levels. Temperature of 30 °C reduced conidial germination of Isolates 7320 and 7771 by 20%, and even less for Isolate 7569.

Tween20 and Tween80 stimulated germination of Isolate 7320 at 25 °C, but inhibited germination of Isolates 7569 and 7771 at the same temperature. Breakthru® showed a similar trend to Tween20 and Tween80 on Isolate 7320. However, Breakthru® caused severe inhibition (< 30% germination) of Isolates 7569 and 7771, even at a temperature of 25 °C.

Discussion

While it is well documented that conidia of entomopathogenic fungi can germinate in surfactants, the viability of conidia may also be influenced by surfactant type (Boucias and Pendland, 1991; Boyette *et al.*, 1996; Milner *et al.*, 1991; Prasad, 1994). This study has shown that not only was conidial germination affected by the surfactant but also by the surfactant concentration and conidial concentration. The three surfactants tested had different effects on the viability of conidia and mycelial growth. However, in both studies, there were enough exceptions to conclude that the concentration should be checked for individual surfactants and individual isolates.

Our study generally showed that it is better to use low or moderate concentrations of surfactants than high concentrations in order to avoid reduced conidial germination. In no case did the use of the three selected surfactants result in improved germination in comparison to applying *B. bassiana* in water. The greatest germination occurred at the lowest surfactant concentrations. Although, *B. bassiana* proved to be an effective biocontrol agent of house flies in our previous studies (Mwamburi *et al.*, 2010), addition of surfactants at low concentrations or by combining the surfactants, the efficacy of *B. bassiana* may still be increased, resulting in improved reliability. Similar observations were noted by Zhang *et al.* (2003). For example, Tween20, which is commonly used for initial screening of different fungi (Boyette *et al.*, 1996), promoted germination at low surfactant concentration of < 1% in Isolate 7320, inhibited germination of 7569 at all concentrations tested and lowered germination at concentrations higher than 0.1% for Isolate 7771. Furthermore, it did not stimulate mycelial growth of any of the *B. bassiana* isolates except for Isolate 7771 at 0.1% concentration. Germination of Isolate 7320 was unaffected by Tween80 up to a concentration of 1% and then decreased significantly at higher concentrations. Isolates 7569 and 7771 were more sensitive to Tween80. Not all of the surfactants were compatible with *B. bassiana* isolates *in vitro*. The Breakthru® series were inhibitory to all the isolates at all concentrations, confirming results of Milner *et al.* (1991), who described toxic effects for various wetting agents.

Tween20 increased mycelial growth of Isolate 7771 at 0.1%, while it decreased mycelial growth of all the other isolates. Tween80 and Breakthru® failed to stimulate mycelial growth of any of the *B. bassiana* isolates. Greater germination was achieved with higher conidial concentrations. This relationship was checked because with similar fungi such as *Colletotrichum spp*., spore germination can be inhibited by high spore densities (Zhang *et al.*, 2003). The observations of higher germination rates with higher conidial densities in this study were consistent with our earlier observations and with other reports related to dose-mortality related studies of *B. bassiana* (Devi *et al.*, 2005; Kaaya and Munyinyi, 1995; Lekimme *et al.*, 2006; Santoro *et al.*, 2008; Watson *et al.*, 1993). In poultry houses and the field, inoculum at high densities are sprayed to target house flies and high germination rates of the inoculum may increase the overall effectiveness of the biocontrol agent.

The optimal temperature for conidial germination of *B. bassiana* isolates was approximately 25 °C, with an upper limit at 30 °C. A temperature of 25 °C was reported to be optimal for *B. bassiana* by Fargues *et al.* (1992). In our study, all isolates showed > 90% conidial germination after 24 h of incubation at 21 °C. Previous studies have shown that *B. bassiana* is mesophilic, capable of growth at a wide temperature range (8-35 °C) with a maximum thermal threshold for growth at 37 °C (Fargues *et al.*, 1997). High temperatures retarded the conidial germination process in *B. bassiana*. Similar delays were found in the same *B. bassiana* isolates (Devi *et al.*, 2005; Luz and Fargues, 1997). This delay is possibly associated with the need to repair damages before germination occurs, as was previously demonstrated in *Bacillus* spores (Nicholson *et al.*, 2000).

Isolate 7320 was identified as the fungal strain that would be most suitable to formulate as a commercial product. Apart from being least affected by the different surfactants, this isolate was able to germinate rapidly in a broad temperature range of 25-30 °C after 24 h, and this characteristic would be a crucial factor in suppressing house flies in poultry houses, where temperatures fluctuate markedly during the day and night. Also, house flies multiply rapidly during the hot summer season; hence, higher germination and growth rates of *B. bassiana* at higher temperatures would be beneficial for house fly control. In addition, studies have also shown that entomopathogenic fungi may experience elevated temperatures through host thermoregulation (Kalsbeek *et al.*, 2001; Olesen, 1985; Watson *et al.*, 1993). These authors reported that infected house flies were capable of elevating their body temperatures through habitat selection or basking in the sun within the first few days of infection and, if the temperatures were high for a sufficiently long period, infected flies would be able to cure themselves from disease (Kalsbeek *et al.*, 2001; Olesen, 1985; Watson *et al.*, 1993).

Our investigation was a laboratory study determining the influences of surfactants upon the first two stages, germination, and mycelial growth, which had some limitations. For example, conidial behaviour was only studied on

agar plates, whereas ultimately infection occurs on an insect cuticle where texture, exudates, and microflora have a role in the pre-infection stages. Also, some surfactants may stimulate host defence responses and thereby reduce disease development in the host (Colson-Hanks and Deverall, 2000). Therefore, studies are recommended to evaluate the effect of these surfactants on pre-infection stages and post-infection disease development on the host house flies.

Acknowledgments

The authors acknowledge Third World Organization for Women in Science (TWOWS) for financial support.

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Associate Editor: Lara Durães Sette