Susceptibility of the German cockroach, *Blattella germanica* to entomopathogenic fungi by using two different methods.

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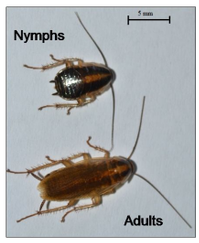
INTRODUCTION

In human residences, cockroaches can contribute to increase allergic processes since they are agents of induction and exacerbation of asthma disease and act as mechanical vectors and natural reservoir of pathogens. Cockroaches are controlled primarily by synthetic organic insecticides. An alternative to chemical methods is the use of entomopathogenic fungi. The overall objective of the present study was to evaluate differential susceptibility of nymphs and adults of *B. germanica* to native isolates of the entomopathogenic fungi *M. anisopliae* and *B. bassiana* comparing between direct contact and bait treatment methods.

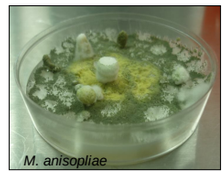
MATERIALS AND METHODS

Pathogenicity evaluation under laboratory conditions on German cockroaches.



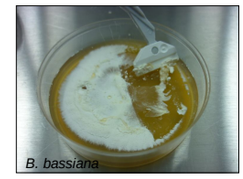
Blattella germanica

Entomopathogenic fungi were selected from the collection of CEPAVE, Buenos Aires, Argentina



Two species of entomopathogenic fungi were evaluated: *Metarhizium anisopliae* (Metschnikoff) Sorokin (CEP 085) strain was originally isolated from an unidentified Hemiptera Cercopidae collected in 2004 from Los Hornos, Buenos Aires, Argentina. *Beauveria bassiana* (Bals. Criv.) Vuil. (CEP 077),was isolated from *Balacha melanocephala* (Hemiptera: Cicadellidae) in 2004 from Los Hornos, Buenos Aires, Argentina

Fungi were tested using two different methods: bait and direct contact.



Fungal cultures: Conidia were harvested from 15 day old cultures incubated at 25ºC. A suspension of conidia in tween 80 (0.01%) was vortexed for 5 min; the concentration of propagules was quantified by using an haemocytometer (Neubauer chamber) and the suspension was adjusted to 1 x 10^9 conidia/ml. Conidial germination percentage was highest of 95 %.

DIRECT CONTACT

Groups of ten cockroaches were exposed to filter papers treated with 1 ml of a suspension containing 1x10^9 conidia per milliliter of *M. anisopliae* or *B. bassiana*. Each filter paper was placed into the bottom of a sterilized Petri dish (100 mm diam). Controls were treated with discs of filter paper (9 mm diam.), with Tween 80, 0.01% (v/v). After 24 h, cockroaches were moved to plastic cups (250 cm3). Food and water were placed inside the containers. Each assay was replicated three times.

BAIT

Bait was prepared with dog food (Purina Dog Chow®, Nestlé Argentina S.A., Buenos Aires) mixed with 1% medium agar water. One ml of the conidial suspension was added to 4 ml of bait, and 4 ml of this mixture was applied on the bottom of 35 mm sterilized Petri dishes. Groups of ten cockroaches were exposed to these baits for 72 h. Then, cockroaches were moved to plastic cups (250 cm3) and maintained with food and water. Each assay was replicated three times.

Mortality was monitored daily for twenty days in both treatment.

RESULTS

Cadavers of cockroaches presented mycelial growth and conidiation in the intersegmental regions when submitted to high humidity conditions.

Adults of *B. germanica* infected with fungi, on the left *B. bassiana* and right *M. anisopliae*.

Table 1 Cumulative mortality (%) of nymphs and adults of *Blattella germanica* exposed to direct contact and baits, with *M. anisopliae* (CEP 085) and *B. bassiana* (CEP 077).

Treatment	Nymphs Days after inoculation					Adults Days after inoculation				
Contact direct	**5 day**	**10 day**	**15 day**	**20 day**	**Sig**	**5 day**	**10 day**	**15 day**	**20 day**	**Sig**
Control	0 ± 0	0 ± 0	0 ± 0	0 ± 0	a	3.3 ± 0.6	10 ± 0	10 ± 0	10 ± 0	a
M. anisopliae	40 ± 1	53.3 ± 0.6	60 ± 1	60 ± 1	b	73.3 ± 2.1	93.3 ± 0.6	93.3 ± 0.6	93.3 ± 0.6	c
B. bassiana	16.7 ± 1.2	40 ± 1.7	40 ± 1.7	40 ± 1.7	b	50 ± 1	80 ± 1	80 ± 1	80 ± 1	bc
Baits	**5 day**	**10 day**	**15 day**	**20 day**	**Sig**	**5 day**	**10 day**	**15 day**	**20 day**	**Sig**
Control	0 ± 0	0 ± 0	0 ± 0	0 ± 0	a	0±0	3.3 ± 0.6	10 ± 1	13.3 ± 0.6	a
M. anisopliae	0 ± 0	0 ± 0	10 ± 1	10 ± 1	a	0±0	23.3 ± 2.1	33.3 ± 3	40 ± 2.6	b
B. bassiana	ND					ND				

For each formulation mean (± standar error); Sig: significance. Different letters on the same column indicate significant differences according to Tukey´s test (P< 0.05). ND: not determined.

Table 2 Median Lethal Time (days) (LT50) of *B. germanica* exposed to direct contact and baits, with *M. anisopliae* (CEP 085) and *B. bassiana* (CEP 077).

Treatment		Nymps				Adults		
Contact direct		**N ª**	**TL_{50}**	**CI 95%***	**Slope(±SE)**	**TL_{50}**	**CI 95%***	**Slope(±SE)**
Ma 085	*B germanica*	60	8.63	3.9 - 27,8	1.29 ± 0.4	3.8	2.5 – 5.43	2.7 ± 0.61
Bb 077	*B germanica*	60	*			4.9	3.1 - 7.7	2.3 ± 0.56
Bait		**N ª**	**TL_{50}**	**CI 95%***	**Slope(±SE)**	**TL_{50}**	**CI 95%***	**Slope(±SE)**
Ma 085	*B germanica*	60	*			*		
Bb 077	*B germanica*	60	ND			ND		

CONCLUSION:

Adults of *B. germanica* were more susceptible to *M. anisopliae* and *B. bassiana* infection than nymphs when direct contact was used; this behaviour was repeated using bait with *M. anisopliae*. Direct contact treatment when compared to bait treatment was more effective.

In our results, the mean survival time range was lower for nymphs and adults of *B. germanica* exposed to surfaces treated by direct contact.

Our results showed that *B. germanica* adults treated with direct contact with *B. bassiana* generated a higher level of mortality which was not significantly different from those insects exposed to *M. anisopliae* with direct contact.

Observed differences between treatments and fungal species tested against cockroaches may be due to the surface structure and the chemical composition of the host cuticle.

We can conclude that *M. anisopliae* CEP 085 and *B. bassiana* CEP 077 strains showed potential as a biological control agent of nymphs and adults of *B germanica.*