Pathogenicity of *Evlachovaea* sp (Hyphomycetes), a new species isolated from *Triatoma sordida*, in Chagas' disease vectors

Patogenicidade de *Evlachovaea* sp (Hyphomycetes), uma nova espécie isolada de *Triatoma sordida*, para vetores da doença de Chagas

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ABSTRACT

Evlachovaea *sp was tested on nymphs of 5* Triatoma *spp 5* Rhodnius *spp,* Panstrongylus herreri *and* Dipetalogaster maximus *at 25°C, 75% humidity and humidity >98%. Most species showed susceptibility to fungal infection at high humidity. Mortality was reduced at 75% humidity. Fungal development was observed on 69.5% of cadavers.*

Key-words: Evlachovaea. *Triatominae. Pathogenicity. Vector control.*

RESUMO

Evlachovaea *sp foi testada em ninfas de 5* Triatoma *spp, 5* Rhodnius *spp,* Panstrongylus herreri *e* Dipetalogaster maximus *a 25°C, 75% umidade e umidade >98%. A maioria das espécies foi suscetível à infecção em umidade alta. Mortalidade foi reduzida a 75% de umidade. O fungo desenvolveu em 69.5% dos cadáveres.*

Palavras-chaves: Evlachovaea. *Triatominae. Patogenicidade. Controle de vetores.*

Entomopathogenic fungi such as *Beauveria bassiana* and *Metarhizium anisopliae* were shown to be active against triatomine bugs under laboratory conditions[2 4]. However, only a few field tests have been reported, and there is almost no published information about pathogenic fungi isolated from field-collected triatomine cadavers. Parameswaran and Sankaran (1979) reported *B. bassiana* on the triatomine bug *Linshcosteus* sp in India. Recently another fungus identified as a new species of *Evlachovaea*, a genus described in Russia[1], was found on a dead *Triatoma sordida* nymph in central Brazil, and its pathogenicity was documented against *Triatoma infestans*[3]. Herein we report results on the pathogenicity of the *Evlachovaea* sp in other triatomine species.

All triatomines tested, 5 *Triatoma* spp, 5 *Rhodnius* spp, *Panstrongylus herreri*, and *Dipetalogaster maximus*, originated from the Institute of Tropical Pathology and Public Health, Goiânia, Brazil. Insects were reared at 25 ± 0.5°C, 75 ± 5% relative humidity (RH), and a photoperiod of 12:12 (L:D) h. They were blood-fed on chickens at 2-week intervals. The fungus was cultivated on complete medium and incubated for 15 days at 25 ± 0.5°C, 75 ± 5% RH, and a photoperiod of 12:12 (L:D) h[3]. For the tests, conidia were suspended in 10ml of sterile 0.1% Tween 80 and adjusted to $3.3x10^6$, 10^7, $3.3x10^7$, 10^8, $3.3x10^8$ and 10^9 conidia/ml, corresponding to between $2.4x10^3$ and $8.0x10^5$ CFU (colony-forming unit)/cm^2 treated surface[4]. Ten recently molted and unfed third instar nymphs (N3) of the various species were directly sprayed with 5ml of each concentration using a Potter spray tower (Burkard Ltd., Hertfordshire, UK). Control insects were treated with 0.1% Tween 80 only. N3 were placed on filter paper in plastic Petri dishes (90 x15mm) and then incubated in a test chamber (33 x 37 x 22cm) at RH 75%, humidity close to saturation (RH>98%), 25 ± 0.5°C and a photoperiod of 12:12 (L:D) h. Humidity of 75% inside the test chamber was regulated with a saturated solution of NaCl. Insects were not fed during the assays. Mortality of nymphs was monitored daily. Lethal concentrations to kill 50% and 90% (LC_{50} and LC_{90}) were calculated by probit analysis. Cadavers were placed in Petri dishes and incubated at RH>98% and 25°C during 20 days.

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Fungal emergence of *Evlachovaea* sp on cadavers and external conidiogenesis were examined daily.

Most triatomine species tested were found susceptible to the *Evlachovaea* sp isolate at RH>98%. Susceptibility of insects was clearly influenced by humidity. At RH 75% mortality was distinctly reduced for all species. Results showed a general relationship between dosage and fungal activity against insects. This was also observed for *T. infestans*[3]. First insects of most triatomine species succumbed to the fungus 5-7 days after application of ≥3.3x10^7 conidia/ml independently of humidity tested. Mortality of *T. vitticeps* and *T. delpontei* initiated after 8 and 9 days incubation at RH>98%, respectively, and the first dead N3 of *T. picturata* exposed to RH 75% were found 12 days after treatment. Total mortality was observed at the highest doses (3.3x10^8 and 10^9 conidia/ml) and humidity close to saturation, except for *T. vitticeps*, *T. picturata* and *T. sordida* which had mortality rates of 10%, 60% and 80%, respectively, 20 days after exposure at RH>98%. Values of LC_{50}, and LC_{90} are presented in Table 1. LC_{50}, 15 days after incubation at RH>98%, varied from 3.18x10^3 CFU/cm^2 (*R. neglectus*) to 4.34x10^5 CFU/cm^2 (*T. sordida*). At RH 75% only *P. herreri* (1.37x10^5 CFU/cm^2) and *R. ecuadoriensis* (3.11x10^5 CFU/cm^2) presented sufficient mortality to calculate the LC_{50}. After 20 days incubation at humidity close to saturation mortality of *T. delpontei*, *R. neglectus*, *R. nasutus* and *D. maximus* were too high to calculate LC_{50} and varied between 2.93x10^3 CFU/cm^2 (*R. prolixus*) and 1.44x10^5 CFU/cm^2 (*T. picturata*) for the other species. At RH 75% LC_{50} 20 days after incubation was 1.12x10^5 CFU/cm^2, 1.68x10^5 CFU/cm^2 and 3.71x10^5 CFU/cm^2 for *P. herreri*, *R. ecuadoriensis* and *D. maximus*, respectively (Table 1). Control mortality at both humidities was ≤10% during the assay, irrespective of the species.

Among all cadavers regardless of triatomine species, humidity or dose tested, 50.8% of the cadavers showed outgrowth of *Evlachovaea* sp only, 18.7% were found with mixed development of *Evlachovaea* sp and other saprophytic fungi,

Table 1 - Lethal concentrations (LC_{50} and LC_{90}) (CFU/cm^2) and respective confidence intervals (95% C.I.) of Evlachovaea *sp. calculated for triatomine third instar nymphs, 15 and 20 days after exposure at 75% and >98% relative humidity and 25°C*[1].

		Time after inoculation (days)			
		15		20	
Species	Humidity (%)	LC_{50} (CFU x 10^5/cm^2)(95% C.I.)	LC_{90}	LC_{50} (CFU x 10^5/cm^2)(95% C.I.)	LC_{90}
Triatoma delpontei	75	*	*	*	*
	>98	0.20^{a,b} (0.05-0.55)	5.02^{b,c} (1.36-402.0)	**	**
Triatoma lecticularia	75	*	*	*	*
	>98	0.89^{b,c} (0.32-2.28)	1.54^{b,c} (1.07-2.61x10^4)	0.20 ^{c,d}(0.12-1.06)	0.52^{a,b} (0.27-279.0)
Triatoma picturata	75	*	*	*	*
	>98	1.66^{c} (0.84-8.30)	9.90^{b,c} (3.27-796.0)	1.44 ^{d}(0.74-5.73)	8.44^{a,b} (2.93-290.0)
Triatoma sordida	75	*	*	*	*
	>98	4.34^{c} (1.74-30)	67.40^{c} (13.80-6.91x10^3)	1.08 ^{d}(0.60-2.69)	6.97^{a,b} (2.79-51.20)
Triatoma vitticeps	75	*	*	*	*
	>98	*	*	*	*
Panstrongylus herreri	75	1.37^{c} (0.69-5.26)	8.77^{b,c} (2.95-235.0)	1.12^{d} (0.51-5.36)	12.3^{a,b} (3.21-682.0)
	>98	0.06^{a} (0.02-0.13)	0.63^{a,b} (0.28-3.89)	0.05^{a,b} (0.02-0.09)	0.27^{a} (0.13-1.64)
Rhodnius ecuadoriensis	75	3.11^{c} (1.29-27.6)	59,30^{c} (11,0-3.4x10^4)	1.68^{d} (0.70-6.66)	33.50^{a,b} (7.85-429.0)
	>98	0.33^{a,b} (0.18-0.55)	0.93^{b} (0.56-4.62)	0.13^{b,c} (0.07-0.21)	0.37^{a} (0.22-1.38)
Rhodnius nasutus	75	*	*	*	*
	>98	0.04^{a} (0.02-0.12)	0.64 ^{a,b}(0.22-4.45)	**	**
Rhodnius neglectus	75	*	*	*	*
	>98	0.03^{a} (0.01-0.06)	0.11 ^{a}(0.06-0.55)	**	**
RRhodnius prolixus	75	*	*	*	*
	>98	0.06^{a} (0.01-0.19)	0.57 ^{a,b}(0.19-6.34)	0.03^{a} (0.01-0.05)	0.08^{a} (0.05-11.1)
RRhodnius robustus	75	*	*	*	*
	>98	0.35^{b} (0.22-0.58)	0.78 ^{a,b}(0.5-2.76)	0.22^{b,c} (0.13-0.44)	0.38^{a} (0.26-15.1)
Dipetalogaster maximus	75	*	*	3.71^{d} (1.02-6.59x10^2)	1.68x10^{2b} (17.0-10^{16})
	>98	0.09^{a} (0.03-0.19)	0.94 ^{a,b}(0.43-4.10)	**	**

[1] five ml suspended conidia at six doses between 3.3x10^6 and 10^9 conidia/ml, corresponding to between 2.4x10^3 and 8.0x10^5 CFU (colony forming unit)/cm^2 treated surface, were applied on 10 recently molted and unfed individuals each using a Potter spray tower. (*) cumulative mortality insufficient, (**) too high to calculate $LC_{50/90}$. Values followed by different letters (a, b, c, d) are significantly different ($P < 0.05$).

25% with unidentified saprophytic fungi, and 5.5% showed no external fungi. Results underline the need to study entomopathogenic fungi for control of triatomine bugs.

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