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Potential of oil-based formulations of *Beauveria bassiana* to control *Triatoma infestans*

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Abstract

The *in vitro* development of *Beauveria bassiana* conidia was monitored when immersed in six concentrations of seven non-ionic (MP 6400, MP 600, Renex 60, Renex 95, Span 80, Tween 20 and Tween 80) and three anionic (DOS 75, Hostapaval BVQ 9 and Surfax 220) surfactants and 11 vegetable oils (linseed, soybean, groundnut, rapeseed, thistle, sunflower, olive, sesame, corn, castor, and babassu). The influence of the oils on the settling behavior of *Triatoma infestans* nymphs and the activity of an oil–water formulation of the fungus against this vector under laboratory and simulated field conditions were also determined. With exception of DOS 75 and Surfax 220 germination of conidia on complete medium was >98% at 24 h after exposure to surfactants up to 10%. Elevated rates of germination (>25%) were observed in 10% corn, thistle and linseed oil 8 days after incubation. Pure oils had a significant repellent effect to *T. infestans*. Repellency decreased generally at 10% of the oil and some oils showed some attractiveness for nymphs when tested at 1%. Nymphs were highly susceptible to oil–water formulated conidia, even at unfavorable moisture for extra-tegumental development of the fungus on the insect cuticle.

Key words: *Beauveria bassiana*, behavior, formulation, surfactant, *Triatoma infestans,* vegetable oil

Introduction

Chagas disease is caused by the protozoan *Trypanosoma cruzi* (Trypanosomatidae) and is a serious health problem in Latin America. Fungi are promising agents for environmentally safe control of triatomines, vectors of this chronic parasitic disease [1–3]. Recent research efforts have concentrated on naturally occurring entomopathogenic fungi in peridomestic habitats, where control with synthetic insecticides is difficult, and their activity against triatomines [4–6]. Preliminary field tests with *Beauveria bassiana* (Hyphomycetes/anamorphic Clavicipitaceae) showed that their use against these vectors can be limited by climatic conditions, especially moisture [2, 7, 8]. Oil-formulated fungi showed good results in biological control of insect pests under field conditions [9–12]. However, there is little information available about the effect of components employed in oil formulations on *B. bassiana* and triatomine vectors. This paper records the effect of adjuvants such as surfactants and vegetable oils on the *in vitro* development of *B. bassiana* and the impact of vegetable oils as a measure of repellency or attractancy on the settling behavior of *Triatoma infestans* (Reduviidae). Moreover, the activity of oil–water formulated conidia of this fungus against *T. infestans* was evaluated under laboratory and simulated field conditions.

Materials and methods

Origin and preparation of the fungus

B. bassiana, CG 14, (Embrapa Genetic Resources and Biotechnology, Brasília, DF, Brazil) was

isolated in 1988 from *Podisus* sp. (Pentatomidae) in Londrina, Paraná, Brazil, and was selected because of its high activity against *T. infestans* [1]. Conidia were obtained from 15-days-old sporulating cultures grown at 25 °C with a photoperiod of 12L:12D h on agar slants of complete medium (CM): 0.001 g $FeSO_4$, 0.5 g KCl, 1.5 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 6 g $NaNO_3$, 0.001 g $ZnSO_4$, 1.5 g hydrolysed caseine, 0.5 g yeast extract, 10 g glucose, 2 g peptone, 20 g agar and 1000 ml distilled water. The isolate was host-passaged before the reported tests on *T. infestans* third instar nymphs in order to stimulate its activity. Conidia were harvested directly by scraping from the surface of the culture. If not mentioned otherwise, inocula were suspended in 10 ml of sterile 0.1% MP 6400, vortexed for 3 min with glass beads, filtered and adjusted to defined concentrations based on haemacytometer counts. Viability of conidia >98% was confirmed at each test. For this suspended conidia were inoculated on CM and germination evaluated 12 h after exposure to culture conditions as mentioned.

In vitro *tests with surfactants*

Seven non-ionic surfactants, MP 6400 (monoleate glycol polyethylene), MP 600, Renex 60 (nonyl phenol), Renex 95, Span 80 (sorbitan monoleate), Tween 20 (polyethylene sorbitan monolaureate) and Tween 80 (polyoxyethylene sorbitan monoleate), and three anionic surfactants, DOS 75 (dioctyl sodium sulfosuccinate), Hostapaval BVQ 9 (nonyl phenol 9 EO sodium sulphate) and Surfax 220 (sodium lauryl sulphate) (all surfactants were obtained from Resinac Indústrias Químicas Ltda, Brazil) were tested. Fifty millilitre of liquid CM without agar were arranged in 100 ml Erlenmeyer flasks and surfactants added at a final concentration of 0.1%, 0.5%, 1%, 2.5%, 5% and 10%. All percentages of surfactants are indicated as vol/vol. A control without surfactant was included. Conidia were inoculated at 10^6 conidia/ml of the medium and flasks incubated at 120 rpm, 25 °C and darkness. Samples of 0.9 ml were taken aseptically for each flask at 0, 4, 8, 12, 16, 20, 24, 48, 72 and 96 h after inoculation, added formol at a final 5% and stored at −20 °C until microscopic observation. Germination kinetics were monitored, considering ungerminated conidia, swollen conidia and germination-initiated conidia with an emerging germ tube shorter than the conidial diameter. Conidia were considered germinated with an elongating germ tube longer than the conidial diameter [13]. Germination rates of four replicates were determined by examination of at least 100 conidia.

Tests with vegetable oils

Eleven commercialized oils extracted from the following plants were tested: linseed, *Linum usitatissimum* (Linaceae) (Naturata, Germany); soybean, *Glycine max*; groundnut, *Arachis hypogaea* (Papilionaceae); rapeseed, *Brassica rapa* (Cruciferae); thistle, *Carduus* sp.; sunflower, *Helianthus annuus* (Compositae); olive, *Olea europaea* (Oleaceae); sesame, *Sesamum indicum* (Pedaliaceae); corn, *Zea mays* (Gramineae) (Rapunzel Naturkost, Germany); castor, *Ricinus communis* (Euphorbiaceae) (Campestre, Brazil) and babassu, *Orbygnia speciosa* (Palmaceae) (both privately manufactured). All oils were obtained from organically grown plants never treated with synthetic pesticides.

Oil–water emulsions were prepared with MP 6400 at 50% of the amount of oil. All percentages of oils are indicated as vol/vol. All components were previously sterilized and then adjusted separately to 80 °C (distilled water) and 60 °C (oils and surfactant) in a water bath. The water was submitted to agitation at 200 rpm and oil and surfactant pipetted simultaneously on the water. The preparation was then agitated during 20 min at ambient temperature and 200 rpm [14].

Conidia were inoculated in 100 ml flasks with 50 ml oil–water emulsions of different oils (10%) at a final 5×10^5 conidia/ml and incubated at 25 °C and 120 rpm. Samples were taken at 0, 24, 48, 72, 96, 120, 144, 168 and 192 h after inoculation and handled as mentioned before. Progress of germination of conidia was examined microscopically using cotton blue at 0.1%.

In a second series of experiments 10^3 conidia/ml were inoculated in emulsified oils at 10% of the oil and incubated at 25 °C and 120 rpm. A control with conidia at the same concentration but suspended only in water was tested. After 24 h incubation 30 μl of the conidial formulate were plated on tetracycline (5 g/1000 ml) amended CM in petri dishes (90 × 15 mm) and incubated at 25 °C and with a photoperiod of 12L:12D h. Development of

CFU (colony forming unit) on the medium was monitored daily during 5 days. Both experiments were repeated four times.

Origin and preparation of insects

Experiments were carried out with laboratory mass-reared *T. infestans*. The colony originated from the State of Paraná, Brazil. Insects were fed on chickens, and maintained in the Institute of Tropical Pathology and Public Health at 25 ± 0.5 °C, 75 ± 5% relative humidity (RH) with a photoperiod of 12L:12D h [15]. For the tests, unfed third instar nymphs (N3) were used. Insects had molted 3 up to 5 days before initiating tests. Nymphs were not fed during the assays.

Tests about settling behavior

Oil–water emulsions were prepared at 1%, 5% and 10% of the oil. Plastic Petri dishes (90 × 10 mm) were subdivided in three areas, A, B and C. For this circular filter paper (90 mm) was cut at the sides into two equal sections of 25 cm^2 and adjusted on the opposite sites of the dish (areas A and C), leaving a stripe of about 1 cm width (area B) without filter paper between A and C. Filter paper of the area A was treated, pipetting 400 μl of pure oil or the emulsions. This quantity was sufficient to impregnate the total section A. Areas B and C were not treated. Lots of 10 N3 were set on area B and dishes transferred to 25 ± 0.5 °C, 75 ± 5% RH and a photoperiod of 12L:12D h. Number of individuals found on the different areas was evaluated during activity (8 p m) and repose (8 a m), 1, 2, 3, 4, 8 and 16 days after exposure. Arrangements of dishes were provided with a camouflage in order to minimize disturbance of the insects during the periodic checks for their position. Permanent indirect light in the darkened room permitted checks to be made during activity of insects without affecting their behavior. The untreated filter paper (area C) was replaced daily at 4 pm in order to reduce contamination from the treated area A with oil or emulsion by moving insects. Assays were repeated four times.

In vivo *tests with fungal formulations*

Insects were treated by directly applying 2 μl of the formulated conidia on the tergal thorax and abdomen using a high-precision micropipette (Eppendorf AG) or indirectly by exposing to previously treated filter paper. Tests were conducted with two formulations, a water (0.1% MP 6400) and oil–water formulation at 5% in the laboratory and 10% of soybean oil in simulated field tests, respectively. Up to six conidial concentrations, 10^5, 3×10^5, 10^6, 3×10^6, 10^7 and 3×10^7 conidia/ml, were assayed. Control insects were treated with 0.1% MP 6400 or emulsion only. Nymphs were aseptically dried for 30 min at 75% RH and 25 °C, placed on filter paper in plastic Petri dishes (90 × 15 mm) and then incubated under laboratory or simulated field conditions in chambers (33 × 37 × 22 cm) with controlled temperature (25 °C), humidity (RH 75% and RH >98%) and photoperiod (12L:12D h) or ambient conditions, respectively. Humidity inside the test chambers was regulated with saturated salt solutions of NaCl (RH 75%) and K_2SO_4 (RH 98%) [16]. Humidity and temperature inside the chamber were measured with a digital thermohygrometer. Preliminary tests showed that humidity was generally reestablished within up to 30 min after opening containers for daily controls of mortality, independently of the ambient humidity and humidities inside the chamber. Four replicates of 10 N3 each were used per treatment. Simulated field conditions were established in small, windowless houses of about 2 × 2 × 2 m built with wooden laths and covered by roofing tiles, located in the proximity of Goiânia, Central Brazil [2]. Tests were done at the beginning of the rainy season in October 2000. Ambient temperature and humidity were registered in a meteorological station of the Federal University of Goiás located at 1 km distance from the experimental houses. Mortality of insects was monitored daily during 15 days. Dead N3 were transferred to a humid chamber and fungal emergence on cadavers recorded.

Analysis of data

The total number of CFU, relative data of germinated conidia, N3 on oil-treated filter paper and fungus-killed insects were analyzed using either one- or two-way analysis of variance (ANOVA) and the Student–Newman–Keuls (SNK) multiple range test of comparison of means. Means were considered not statistically different at $P > 0.05$. Probit analysis for determination of LD_{50}, LD_{90}, LC_{50}

and LC_{90} were conducted on total mortality after Abbott transformation using log-probit analysis [17].

Results

Effect of surfactants on germination

First swollen and germination-initiated conidia were observed 4 and 8 h after exposure to surfactants, respectively. A highly significant effect of the surfactants ($42.6 \leq F_{9;39} \leq 128.3$; $P \leq 0.003$) and concentrations tested ($4.9 \leq F_{6;27} \leq 971.4$; $P < 0.001$) on the germination was found at a 12 h incubation (Table 1). Mean germination of conidia in CM without surfactant varied between 15.5% and 21% ($F_{9;39} = 1.4$; $P = 0.22$). Testing surfactants at 0.1%, germination varied between 0.3% (Surfax 220) and 19.5% (Tween 80) and between 0% (DOS 75, Span 80 and Surfax 220) and 9% (Hostapaval BVQ 9) at 10% of the surfactants, respectively, at this moment (Table 1). After 24 h exposure germination of conidia was >98% for all surfactants except DOS 75 and Surfax 220. For DOS 75 germination of 64.5 ± 1.4% was observed at 0.1% of the surfactant. At higher concentrations of DOS 75 germination was significantly inferior ($F_{6;27} = 877.9$; $P < 0.001$) and varied between 26.3 ± 0.8% and 19.5 ± 0.3% at 0.5% and 5% of the surfactant, respectively. Testing the same surfactant, germination 96 h after incubation was highest (>98%) at 0.1% and dropped to 48.5 ± 0.7% at 5% of the surfactant. All (>98%) conidia had germinated at 0.1% Surfax 220 24 h after incubation but no germination of conidia was found testing concentrations ≥0.5% at this moment. Germination reached 13 ± 2.6% and 2.5 ± 1.3% after exposure to Surfax 220 at 0.5% and 1% during 96 h, respectively. At higher concentrations (2.5–10%) conidia did not swell nor initiate germination.

Effect of vegetable oils on B. bassiana

Germination of conidia, 24 h after incubation in 10% oil–water formulations varied between 0% (on babassu, groundnut, linseed, sesame, sunflower and thistle) and 2.5 ± 1.6% (on corn) (Table 2). After a 4 day incubation in the emulsions, germination rates ≤1.3% were found for 72.2% of the oils tested. Only soybean (9.5 ± 1%), thistle (11.5 ± 1%) and corn oil (64.3 ± 1.9%) induced elevated germination. Relative numbers of germinated conidia 8 days after incubation varied between 4.8 ± 0.3% (sunflower) and 92.5 ± 1.2% (corn). There was a significant effect on the germination of *B. bassiana* conidia of the oil tested after 1 day ($F_{10;43} = 2.4$; $P = 0.03$), 4 days ($F_{10;43} = 594.6$; $P < 0.001$) and 8 days ($F_{10;43} = 951.9$; $P < 0.001$) (Table 2).

Conidia incubated for 24 h in oil–water emulsions at 10% of the oils developed to CFU with numbers varying between 25 ± 2.7 CFU (linseed) and 42 ± 1.8 CFU (soybean). The number of CFU obtained with conidia incubated in water only was 25.5 ± 0.6. The number of CFU found differed significantly among oils tested ($F_{10;43} = 5.5$, $P < 0.001$) (Table 2).

Effect of oils on settling behavior

No mortality of *T. infestans* was observed during the assays, independently of the oil or its concentrations tested. Relative numbers of N3 on oil-treated filter papers are shown in Figure 1. Area selection of *T. infestans* nymphs depended on different oils tested ($F_{10;87} = 5.3$, $P < 0.001$). The number of nymphs found on oil-treated filter papers, increased according to the oils, regardless of the concentration tested: babassu, groundnut, corn ≥ castor ≥ olive, thistle, rapeseed, soybean ≥ sunflower and linseed oil. Results obtained with sesame oil did not differ significantly from other oils. Results also showed a clear effect of oil concentration on the selection behavior of N3, independently of activity or repose ($F_{3;87} = 30.6$, $P < 0.001$). The number of N3 on oil-treated filter paper at 1% of the oil was 55.7%, in activity or repose, with highest values ≥75% found for castor, rape seed and thistle, and decreased to 49.4% and 36.6% at 5% and 10% of the oil, respectively. A distinctly reduced number of N3 with only 24% was found when insects were exposed to substrates treated with pure oil (Figure 1). No significant difference of nymphal behavior during activity or repose was detected ($F_{1;87} = 0.99$, $P = 0.48$). During 16 days of observation insects did not modify area selection in repose ($F_{5;43} = 0.71$, $P = 0.61$), but the number of N3 on oil-treated filter paper increased significantly, when checked during insect activity, 8 and 16 days after initial exposure ($F_{5;43} = 2.5$, $P = 0.03$).

Table 1. Germination (%) of *Beauveria bassiana*, CG 14, 12 h after exposure to surfactants at different concentrations*

Surfactant	Concentration (%) (vol/vol)							$F_{6,27}$ values	Significance
	0	0.1	0.5	1	2.5	5	10		
DOS 75	19.8 ± 0.7 a1a2	1 ± 0 b1d2	0 c1e2	0 c1d2	0 c1d2	0 c1d2	0 c1, d2	971.4	$P < 0.001$
Hostapaval	18.5 ± 2.3 a1a2	17.8 ± 1 a1a2	17.5 ± 2.2 a1a2	18 ± 1.8 a1a2	19.5 ± 1.3 a1a2	15 ± 2 b1a2	9 ± 0.5 b1, a2	4.9	$P = 0.003$
MP 6400	15.5 ± 1.6 a1a2	1.3 ± 0.3 b1d2	2.8 ± 0.5 b1c-e2	0.8 ± 0.2 c1d2	1 ± 0 b1d2	1.3 ± 0.7 b1d2	0.8 ± 0.3 c1, d2	63.5	$P < 0.001$
MP 600	15.5 ± 1.4 a1a2	1.5 ± 0.7 b1d2	1 ± 0.5 b1de2	2 ± 0.5 b1d2	1 ± 0.5 b1d2	0.5 ± 0.3 b1d2	0.6 ± 0.3 b1, d2	74	$P < 0.001$
Renex 60	16.5 ± 1.5 a1a2	11.5 ± 1.2 b1b2	12.5 ± 1.4 b1b2	11.5 ± 0.7 b1b2	6 ± 0.4 c1c2	2 ± 0.7 d1c2	0.3 ± 0.3 d1, d2	37.2	$P < 0.001$
Renex 95	18.3 ± 2 a1a2	6 ± 0.4 b1c2	4.3 ± 0.5 bc1cd2	5.5 ± 0.7 b1c2	6 ± 0 b1c2	3 ± 0.4 bc1c2	2 ± 0.4 c1, c2	41	$P < 0.001$
Span 80	17 ± 1.6 a1a2	18 ± 1 a1, a2	6 ± 1.5 b1c2	1 ± 0.4 c1d2	1.5 ± 0.3 c1d2	0 c1d2	0 c1, d2	77.5	$P < 0.001$
Surfax 220	17.3 ± 2 a1a2	0.3 ± 0.3 b1d2	0 b1e2	0 b1d2	0 b1d2	0 b1d2	0 b1, d2	78.1	$P < 0.001$
Tween 20	15.8 ± 1.5 a1a2	4.5 ± 0.3 b1c2	1.5 ± 0 c1de2	2.3 ± 0.9 c1d2	1 ± 0.4 c1d2	1 ± 0.4 c1d2	0.3 ± 0.3 c1, d2	59.4	$P < 0.001$
Tween 80	21 ± 0.6 a1a2	19.5 ± 1.5 a1a2	16 ± 0.8 b1a2	12.3 ± 1 c1b2	9.3 ± 0.5 d1b2	8 ± 0.7 de1b2	5.8 ± 0.5 e1, b2	48.4	$P < 0.001$
$F_{9,39}$ values	1.4	99.2	48.3	65.7	128.3	42.6	113		
Significance	$P = 0.22$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$		

*Conidia were inoculated to complete medium at a final 1×10^6 conidia/ml and incubated at 25 °C and 120 rpm. Mean values of four different replicates with counts of at least 100 conidia per sample with its respective standard error of the mean. Means within the same row (1) or column (2) followed by different letters (a, b, c, d, e) are significantly different ($P < 0.05$) according to the SNK test.

Table 2. Effect of vegetable oils on the *in vitro* development of *Beauveria bassiana*, CG 14

Vegetable oil	Number of CFU *	Germination rates (%) after specified number of days **		
		1	4	8
Babassu	27 ± 2.4 b–d	0 b	0.3 ± 0.3 c	5.3 ± 0.3 f
Castor	31.8 ± 1.1 a–d	0.5 ± 0.3 ab	0.5 ± 0.3 c	5 ± 0.4 f
Corn	36 ± 2.5 a–c	2.5 ± 1.6 a	64.3 ± 1.9 a	92.5 ± 1.2 a
Groundnut	35.3 ± 1.1 a–d	0 b	0 c	10.3 ± 0.6 e
Linseed	25 ± 2.7 d	0 b	0 c	27 ± 1.5 c
Olive	29 ± 1.9 b–d	0.3 ± 0.3 ab	1 ± 0.4 c	5 ± 0.4 f
Rapeseed	36.5 ± 3.6 ab	0.3 ± 0.3 b	1.3 ± 0.6 c	12.3 ± 0.9 e
Sesame	40.3 ± 4 a	0 b	1 ± 0.4 c	11.3 ± 0.5 e
Soybean	42 ± 1.8 a	1.3 ± 0.3 ab	9.5 ± 1 b	19 ± 1.1 d
Sunflower	31.5 ± 1.7 a–d	0 b	0.3 ± 0.3 c	4.8 ± 0.3 f
Thistle	32.8 ± 2.1 a–d	0 b	11.5 ± 1 b	32.8 ± 1 b

* Conidia were incubated in oil–water emulsion at 10% (vol/vol) of the oil during 24 h at 25 °C and 120 rpm, then inoculated on complete medium and incubated at 25 °C during 5 days. Mean values of CFU (colony forming unit) of four replicates with its respective values of standard error of the mean (SEM).

** Conidia incubated in oil–water emulsion at 10% of the oil (vol/vol) at 25 °C and 120 rpm. Mean values of four different replicates with counts of at least 100 conidia per sample (SEM).

Means within the same column followed by different letters (a, b, c, d, e, f) are significantly different ($P < 0.05$) according to the SNK test.

Laboratory tests about the effect of oil-formulated conidia in T. infestans

Direct application of oil–water formulated conidia on insects induced a significant dose-related mortality among N3, which varied between 5 ± 0.3% and 85 ± 0.3% at 10^5 and 3×10^7 conidia applied ($F_{5;23} = 9.3$; $P < 0.001$) 15 days after incubation at 75% RH and 25 °C. At this moment a dose of 636 (I. C. 427–952) conidia on each N3 was sufficient to kill 50% of the nymphs at 15 days of incubation (Table 3). Testing water-formulated conidia ≥75% of N3 had survived when treated directly. Significantly more conidia were necessary to kill 50% of the N3 treated indirectly with an aqueous formulation of conidia (1.16×10^5 conidia/cm^2), compared to the oil–water formulated conidia (5.26×10^4 conidia/cm^2) (Table 3).

Simulated field tests

Ambient temperature and relative humidity, monitored for 15 days, varied between 24.5 and 35 °C and 74 up to 97% RH, respectively. Moderate precipitations and RH > 90% were registered only in the last 2 days of the experiments. Cumulative daily insolation varied between 4.2 and 9.2 h. First dead N3 were found 3 days after treatment. Cumulative mortality 5 days after exposure varied between 20 ± 4.1% (6×10^3 conidia/individual at RH > 98%) and 45 ± 8.7% (2×10^4 conidia/individual at ambient humidity) and did not exceed 2.5% among insects treated with water-formulated conidia, independently of the concentration and humidity tested. The number of mycotized nymphs then increased quickly, especially at RH > 98% (Figure 2c, d) and within N3 treated with oil–water formulated conidia and exposed to ambient humidity (Figure 2b). Most nymphs treated with the aqueous formulation had survived 15 days after treatment and exposure to ambient humidity except at the highest concentration (6×10^4 conidia/individual), where 45 ± 9.6% of the N3 were found dead (Figure 2a). Values of LD_{50}, 10 days after treatment with oil–water formulated conidia did not differ among humidities tested, 331 (I.C. 64–757) conidia/N3 at ambient RH and 50.9 (I.C. 0.22–275) conidia/N3 at RH > 98%, but were significantly different from the LD_{50} found for

Figure 1. Settling behavior of *Triatoma infestans* third instar nymphs (N3) on oil-treated substrates: means (%) (standard error of the mean) of insects observed in activity and repose, up to 16 days after exposure on filter paper treated with 1%, 5%, 10% oil–water emulsions (vol/vol) and pure 11 different vegetable oils, related to the total number of insects.

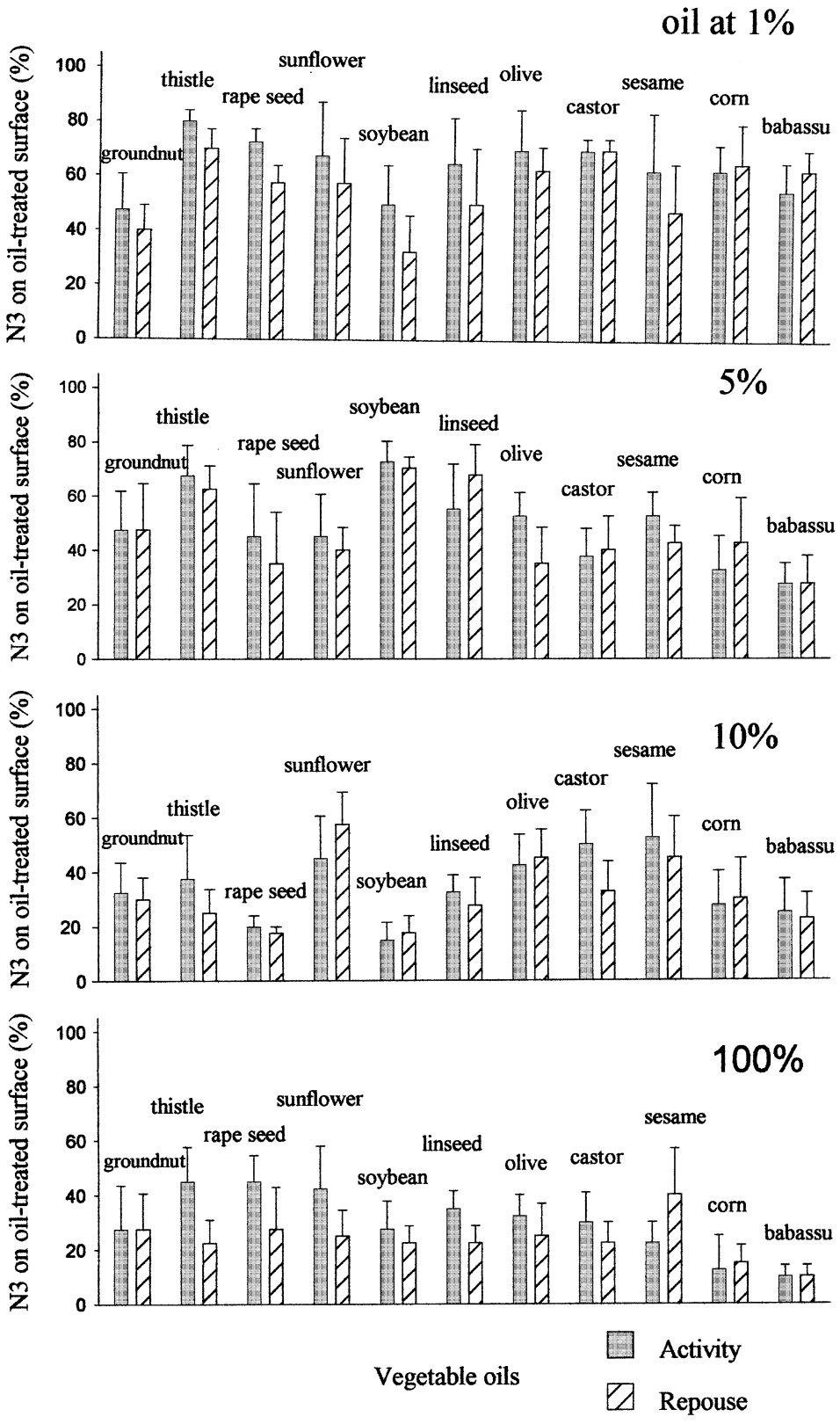
oil at 1%
5%
10%
100%
N3 on oil-treated surface (%)
0
20
40
60
80
100
groundnut
thistle
rape seed
sunflower
soybean
linseed
olive
castor
sesame
corn
babassu
Vegetable oils
Activity
Repouse

Table 3. Lethal doses (LD_{50} and LD_{90}) (conidia/individual) and concentrations (LC_{50} and LC_{90}) (conidia/cm^2) with respective confidential intervals (95% C. I.) and slopes (±standard error) calculated for *Triatoma infestans* third instar nymphs, treated directly and indirectly with water and oil–water formulated conidia of *Beauveria bassiana*, CG 14, 15 days after exposure to 25 °C and 75% relative humidity *

Application	Formulation	LD/LC_{50} (95% C. I.)	LD/LC_{90} (95% C. I.)	Slope ± SE
Conidia/Individual **				
Direct	Aqueous	***	***	–
	Oil–water	6.36×10^2 (4.27×10^2–9.52×10^2)	6.39×10^3 (3.28×10^3–2.18×10^4)	0.99 ± 0.11
Conidia/cm^2 ****				
Indirect	Aqueous	1.16×10^5 (9.39×10^4–1.48×10^5) a	2.99×10^5 (2.2×10^5–4.79×10^5) a	1.15 ± 0.12
	Oil–water	5.26×10^4 (4.34×10^4–7.53×10^4) b	2.27×10^5 (1.53×10^5–4.1×10^5) a	2.11 ± 0.25

* Soybean oil water formulation at 5% of the oil (vol/vol) with six concentrations between 1×10^5 and 3×10^7 conidia/ml was tested with four replicates of 10 nymphs each. Means of $LC_{50/90}$ among the same application followed by different letters (a, b) are significantly different.
** Nymphs were topically treated with 2 μl of the formulate.
*** Cumulated mortality insufficient to calculate values of $LD_{50/90}$.
**** Filter paper was treated with 1 ml of the formulate and nymphs exposed to dried support.

nymphs treated with water-formulated conidia and exposed to RH > 98% (Table 4). *B. bassiana* developed on all dead N3 after transfer to a humid chamber. No mortality of water or oil–water treated control nymphs was detected during the assays.

Discussion

The results clearly showed a retarding or inhibitory effect of all non-ionic and anionic surfactants on initial germination of *B. bassiana* conidia after exposure to ≥5% surfactant added CM. However, no more effect on germination was observed after 24 h incubation, except for DOS 75 and Surfax 220, which are both anionic surfactants. Surfactants are important compounds of oil and aqueous formulations and are used at higher concentrations in emulsions depending on the oil fraction in the formulation. Combinations of non-ionic and/or ionic surfactants may be desirable in order to produce more stable mycoinsecticides with enhanced activity, than may be possible with only a single surfactant. Non-ionic surfactants are generally considered to be less toxic than ionic surface active agents. The toxicity of surfactants to microorganisms is related to membrane-damaging effects and may depend on the alkyl chain length. Motility of the protozoan *Tetrahymena elliotti* (Tetrahymenidae) was inversely affected by increasing hydrophilicity of non-ionic surfactants [18]. In the present study Surfax 220 obviously did not interfere with germination at the lowest concentration (0.1%) tested, but germination of conidia was severely affected at higher concentrations (0.5% and 1%) and completely inhibited at concentrations ≥2.5% after up to 96 h of exposure. DOS 75 was also harmful for conidial germination at concentrations ≥0.5%, but values of germination within 96 h reached at least 48.5% independently of the concentration tested. Interestingly, the third anionic surfactant tested, Hostapaval BVQ 9, did not affect germination at even the highest concentration (10%) tested. Garon et al. [19] tested two non-ionic (Triton X-100 and Tween 80) and one anionic surfactant (SDS) on 18 different fungal species and observed a good tolerance of both non-ionic surfactants and a distinct growth inhibition after exposure to the anionic surfactant. Non-ionic surfactants may be detrimental for fungal development. For example, Tween 20 and Tween 80, are commonly used to suspend lipophilic conidia but both surfactants significantly reduced mycelial growth of the phytopathogenic fungus *Chondrostereum purpureum* (Meruliaceae) after inoculation on surfactant (0.1%) amended malt–dextrose–agar medium [20]. Surfactants may also have a stimulatory effect on fungi. Abbas and Egley [21] found up to 5% germinated conidia of *Alternaria helianthi* (Pleosporaceae) after exposure of conidia to 0.2% Tween 20 and Tween 80 during 96 h at temperatures between 18 and 28 °C. Moreover, primary conidia of *Erynia delphacis* and *E. neoaphidis*

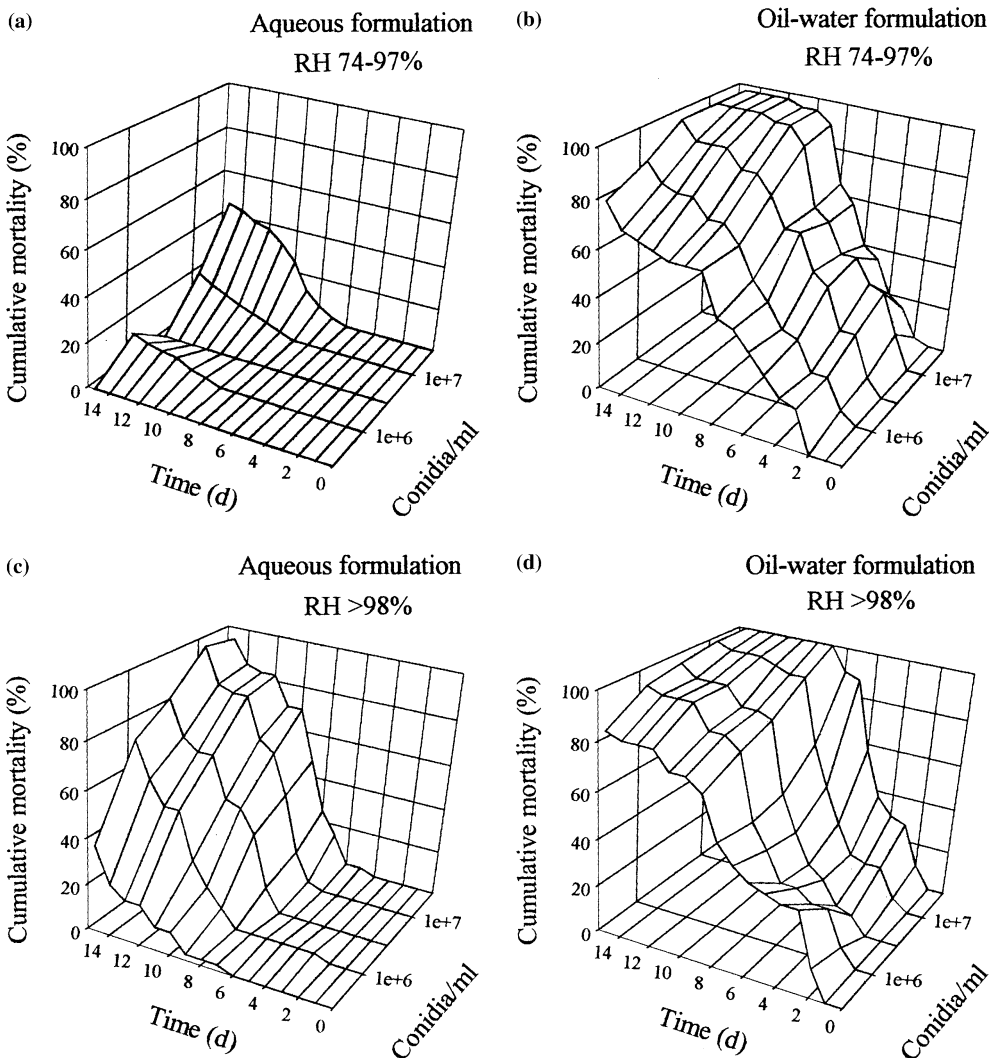

Figure 2. Cumulative mortality (%) of *Triatoma infestans* third instar nymphs, after direct application of water-formulated (a, c) and soybean oil–water formulated (10%, vol/vol) (b, d) *Beauveria bassiana*, CG 14, conidia and exposure at semi-field conditions to ambient temperature (24.5–35 °C) and humidity (RH 74–97%) (a, b) or relative humidity close to saturation (RH > 98%) (c, d).

Table 4. Lethal doses (LD_{50} and LD_{90}) and respective Confidential Intervals (95% C. I.) and slopes (±standard error) demonstrated as conidia / *Triatoma infestans* third instar nymphs after direct treatment with water and oil–water formulated conidia of *Beauveria bassiana*, CG 14, and exposure for 10 days to ambient humidity (74–97%), RH >98% and ambient temperature 24.5–35 °C under simulated field conditions *

Formulation	Humidity (%)	LD_{50} (95% C. I.)	LD_{90} (95% C. I.)	Slope ± SE
Aqueous	74–97	**	**	–
	>98	1.37×10^4 (8.46×10^3–2.53×10^4) a	3.51×10^5 (1.27×10^5–2.31×10^6) a	0.97 ± 0.19
Oil–water	74–97	3.31×10^2 (64–7.57×10^2) b	1.09×10^4 (5.79×10^3–3.16×10^4) b	0.87 ± 0.29
	>98	50.9 (0.22–2.75×10^2) b	8.12×10^3 (3.28×10^3–4.14×10^4) b	1.06 ± 0.38

* Nymphs were topically treated with 2 μl of the soybean oil formulate at 10% of the oil (vol/vol). Six final concentrations between 6×10^2 and 6×10^4 conidia/individual were tested with four replicates of 10 nymphs each. Means of $LC_{50/90}$ among the same column followed by different letters (a, b) are significantly different.

(Entomophthoraceae) incubated in 0.1% Tween 20 added Entomophthora-complete-medium formed germ tubes, due to a probable catalytical action or by increasing hydratation of conidia; resporulation on Tween 20 added non-nutrient media was observed, but both fungal species did not form hyphae [22].

Results showed that *B. bassiana* conidia may initiate germination after exposure to emulsified vegetable oil and that the kind of oil has a decisive influence on quantitative germination. Conidia germinated best with corn oil (92.5%), followed by thistle (32.8%), linseed (27%) and soybean oil (19%), at 10% of the oil. Qualitative and quantitative composition of fatty acids which are components of vegetable oils, and surfactants as well as of the insect epicuticle were shown to affect fungal development. Whereas long chain fatty acids obtained commercially or from epicuticular lipids, such as myristic, oleic and palmitic acid stimulated germ tube formation and resporulation of *Erynia* spp. [22], they were shown poor sources of carbon and energy for *B. bassiana* development under *in vitro* conditions: more than the half of conidia did not develop beyond swollen conidia [23]. Linoleic acid which can be found in high proportions ($>50\%$) in corn, soybean and thistle oil had a fungistatic effect on *Erynia* spp. [22], but did not prevent growth of *B. bassiana* in the present study. Interaction between fatty acids and other components of the oil and their concentration certainly do have either a stimulating or inhibitory effect on fungal development. There was obviously no toxic effect of all vegetable oils tested at 10% on *B. bassiana*, as values of CFU were comparable or even higher than values obtained with conidia suspended in surfactant added water. The higher number of CFU obtained with emulsions may be related to a stimulatory effect of the oil or a better dissociation of conidia in the oil water formulation, compared to water-suspended conidia.

Nymphal survival was not affected after exposure to oil-treated substrates. Toxicity of vegetable oils to insects which depends on composition, formulation and application of the oils and susceptibility of the target insect has been reported against *Sitophilus oryzae* (Curculionidae) with sesame oil [24] or against aphids with soybean and undefined vegetable oils [25–28]. Vegetable oils may have a repellent effect against other insects such as aphids and pests of stored seeds and grains [29–33]. However, repellency of vegetable oils was reduced for mosquitoes in comparison with essential oils [34]. Results demonstrated that vegetable oils tested interfere with the settling behavior of *T. infestans*. Fungal formulations based on vegetable oils may attract this vector at reduced concentrations of the oil, but areas treated with high oil-dosed formulations may be avoided.

Results obtained about activity of formulated conidia against *T. infestans* in laboratory and simulated field tests demonstrated that both formulation and application techniques affected infection rates. Activity of *B. bassiana* in triatomines was closely related to the dose and relative humidity. Highest susceptibility to *B. bassiana* was found at humidity close to saturation. The dependence of this and other entomopathogenic fungi on high humidity for infection of triatomines had been reported previously under laboratory conditions. In these studies higher conidial doses also compensated for unfavorable moisture [1, 4, 5, 35, 36]. We observed a clear difference between activity of water- and oil-formulated conidia against nymphs. After adding oil to the formulation more insects succumbed to the infection than when exposed to water-formulated conidia, even at unfavorable conditions of humidity. Quantitative contamination of insects was highest when applying oil-formulated conidia directly onto the insects, due to a better adhesion and spreading of the formulation on the lipophilic cuticle than for conidia formulated in water only. Moreover, oil which formed a film on the cuticle acted as an antievaporant and the surfactant served as a humectant (since their hydrophilic component helps to hold moisture); both, then, contributed to establish favorable conditions for germination and invasion of an elevated number of conidia. Tests about indirect applications by exposing nymphs to previously treated filter paper showed that insects were contaminated and successfully infected by contact with the conidia-treated filter paper. Quantitative contamination by conidia, especially of the legs and ventral body parts was probably inferior compared to direct application, thereby explaining the higher LC_{50} values found in the indirect treatment tests. Conidia formulated in water only were less effective under laboratory and

simulated field conditions, regardless of the application technique used.

Oil–water formulated conidia with adjuvants added as a tank mix to conidia before application, seemed to be the most adequate formulation to infect insects directly in their peridomestic hiding places. An elevated volume of water applied by high pressure equipment will increase a direct contact of the formulation with the insect and elevate temporarily moisture to a favorable level for fungal development in the microhabitat. Recent field tests with CG 14 in peridomestic areas infested with *T. sordida* in Central Brazil underlined the utility of oil-based formulations to control triatomine vectors [8]. Further tests of improved formulation and application techniques may open new possibilities to contribute to an effective and permanent control of peridomestic triatomine species which continue to be a risk as vectors of human Chagas disease.

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