Research Article

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Biological control of *Cimex lectularius* with *Beauveria bassiana*: Effects of substrate, dosage, application strategy, and bed bug physiology

Anders Aak, Morten Hage and Bjørn Arne Rukke*

Abstract

Background: *Cimex lectularius* L. (bed bug) (Hemiptera: Cimicidae) is a serious indoor pest worldwide, and this nuisance needs to be controlled using different methods in integrated pest management (IPM). *Beauveria bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae) kills bed bugs, and insect pathogenic fungi may be utilized to control bed bugs in IPM. To increase knowledge of this methodology, forced exposure experiments were conducted with different formulations, doses, and substrates, using bed bugs in variable physiological states.

Results: Both oil- and water-formulated fungal products showed significant improvement when conidial concentrations were raised in five steps from 0.02 to 2.0%. At low concentrations (0.02% in water) effects from substrate and application strategy were observed. Application on soft substrates (cotton and polyester) yielded significantly higher bed bug mortality rates than on harder substrates (paper, wood, and linoleum) with a final mortality of 35–63% against 8–10%. Multiple applications over time also improved *B. bassiana*'s ability to kill bed bugs, and at low concentrations only a triple application on cotton showed 100% final mortality. Bed bug age and reproductive status significantly affected survival. Older and reproducing individuals showed higher mortality compared to newly emerged adults. Differences in feeding status also yielded differences in mortality timing, but only minor differences in final mortality rates. Egg production and hatching success were significantly reduced by some treatments.

Conclusion: *B. bassiana* appears to be an asset in the fight against bed bugs. Substrate, dosage, application strategy, and bed bug physiology are important factors to consider for optimal efficacy and safe indoor control with insect pathogenic fungi.

Keywords: insect pathogenic fungi; bed bug; substrate; physiology; IPM; dose–response

1 INTRODUCTION

Insect pathogenic fungi can be used to kill various insects.[1,2] Their ability to decimate insect pest populations depends on their affinity for the host and the magnitude of transmission within the population. Population impact is also regulated by biotic and abiotic factors in the environment, which produce the desired control effect when combined with the appropriate formulation and application regimens.[3–5] The primary use of biological control methods is found in ecoagricultural systems and greenhouses, but insect pathogenic fungi may also facilitate indoor pest management, for example *Cimex lectularius* (bed bug) control.[6–8]

Among the easily obtainable fungal species, *Beauveria bassiana* is reported to be a strong candidate because it is fully functional and supplies horizontal transfer between aggregating individuals in the quite dry conditions expected to be found in bed bug habitats.[7,8] Dried substrates with conidia and dry conidia infect bed bugs, and are capable of killing pesticide-resistant bed bug strains.[6,9] *Metharizium anisopliae* also can infect and kill bed bugs, but requires high levels of moisture to do so, whereas *Cordyceps fumosorosea* and *Akanthomyces muscarius* do not cause mortality at low doses in dry environments.[8,10] Apart from fungi, it is believed that no other biological control agents exist that are suitable for efficient and efficacious indoor use against bed bugs.[11,12]

B. bassiana appears to kill bed bug juvenile stages and adults equally well, but the potential impacts of variations in other physiological factors and the environment have only been partially investigated.[7,13,14] The conidia are expected to remain viable for several weeks indoors, but bed bug aggregation chemicals may negatively influence the conidial functionality.[15–18] Temperature stress due to increased ambient temperatures may increase the effect of insect pathogenic fungi on bed bugs, and ticks have shown variation in mortality associated with feeding status or developmental stage.[19–21] Environmental factors are distinct determinants of the effect of insect pathogenic fungi outdoors.[4,17]

* *Correspondence to: BA Rukke, Department of Pest Control, Norwegian Institute of Public Health, Lovisenberggata 8, Pb. 4404 Nydalen, NO-0456 Oslo, Norway, E-mail: bjornarne.rukke@fhi.no*

Department of Pest Control, Norwegian Institute of Public Health, Oslo, Norway

Describing the influence of as many relevant factors as possible is therefore important to fully understand the practical use of insect pathogenic fungi against bed bugs. This may allow the development of more efficient and effective indoor application approaches, and pave the way for high-quality tests on bed bug infestations in apartments.

Bed bugs are obligate blood feeders in all development stages and use humans as their main host. They disperse by hitching rides on objects such as suitcases, bags, boxes, and furniture, and typically establish in bedrooms at their new locations. This nuisance has been kept at bay using conventional chemical treatment since the 1950s, and the need for the development of biological control methods has been limited. However, increased travel, along with widespread resistance against commonly used pesticides (e.g., pyrethroids, organophosphates, neonicotinoids, and organochlorines) has allowed bed bugs to make a significant resurgence as a nuisance pest worldwide.[11,22] Their economic impact is substantial, and bed bugs cause both direct costs through control efforts and indirect costs through reputational loss or by tying up resources in the management of apartment buildings, businesses, organizations, and public services.[23–25] This has called for the full extent of integrated pest management (IPM) strategies which rely on combinations of several methods to prevent and eradicate infestations.[25–28] Insect pathogenic fungi are comparable with traditional chemical pesticides because they can be formulated as liquids for spray application in bed bug infested rooms. The efficient use of pesticides requires knowledge of lethal dosage, residual effects, application regimen, environmental interactions, and safety considerations.[29,30] These aspects also apply to *B. bassiana* because elevated indoor conidia levels may result in allergic reactions or exacerbate asthmatic and respiratory symptoms in people.[31–34] A balance between efficiency and safety can be attained through limited use, reduced concentration of harmful substances, or strategic application to safeguard inhabitants during and after the application of IPM measures.

This study investigates and describes the effects of relevant substrates, inoculation dosages, application methods, and the influences of blood feeding, age, and reproduction on *B. bassiana* performance as a bed bug control. The subordinate consequences for female fecundity during an ongoing fungal infection are also described to allow a more extensive discussion of potential field effects.

2 MATERIALS AND METHODS

2.1 Bed bugs

Bed bugs in stock cultures were originally collected in 2009 from two hotels in Oslo, Norway (59°54′49.9284″ N, 10°45′8.082″ E). The bed bugs were fed 37 °C human blood through a parafilm membrane.[35] Fifth-instar nymphs were provided with a bloodmeal, and newly emerged adults appeared after 10–14 days. The adult bed bugs were included in experiments within the following week. This ensured that <10 days had passed following molting before bed bugs were assigned to the various experiments. Bed bugs from different feeding batches and stock culture cohorts were evenly distributed across all different experiments. Stock cultures and experimental insects were kept at 22 °C in climate chambers (Sanyo-MLR-351H, Medinor ASA, Oslo, Norway) with a 16:8 h (light/dark) cycle and 60% relative humidity before fungal exposure. The laboratories in which the fungi exposure and mortality observations took place maintained an equal light cycle, a temperature of 22–23 °C, and a relative humidity of 40–50%.

2.2 Insect pathogenic fungi

Two commercially available products which were previously reported to kill bed bugs were chosen for the experiments.[8] BotaniGard 22WP (*B. bassiana* strain GHA, 2×10^{13} cfu/kg; Laverlam International, Butte, MT, USA) was prepared following the manufacturer's instructions by dissolving the product in water and diluting to the recommended concentration of 0.02%, and Aprehend (ConidioTech-*B. bassiana* strain 193–825; Pennsylvania State University, State College, PA, USA) was a ready-to-use, 2.0% conidia solution dissolved in oil (petroleum distillates). Conidial viability was confirmed according to the growth protocol for water or oil preparations, and germination percentages were found to be 64% and 78% for water and oil, respectively.[36]

2.3 Experimental protocol

2.3.1 *Application and exposure*

Circular substrates, cut out to fit the bottom of the containers (diameter = 47 mm), were used to expose bedbugs to conidia. According to the requirements of the experiment, conidia were applied by either dipping these substrates in the conidial suspension to get a complete coverage or by spraying them with a handheld pump sprayer to approximate a field application on various substrates (Gloria 0.5 L Fine sprayer, Profiline 88; VWR, Oslo, Norway). Dipping resulted in 6.9×10^5 and 5.3×10^7 conidia/cm^2 for the water- and oil-based products, respectively (experiments 1, 3, and 4). The approach that inoculated fungal formulations on different substrates using a fine sprayer was not precise enough to allow estimation of conidia density (experiment 2). Substrates with water- and oil-based products were kept at room temperature until the surface appeared dry when touching it before being used (3–4 days for water, 10–12 days for oil). Inoculated substrates were placed on the bottom of plastic experiment boxes (140-mL straight sample containers; VWR), and the smooth polyethylene surfaces ensured that the bed bugs held onto the substrate and remained on it throughout the exposure duration.

2.3.2 *Control insects*

Bed bugs were kept on clean filter paper to provide 180 bed bugs in 30 boxes without exposure to *B. bassiana* (control). Additionally, 48 adult bed bugs distributed in eight boxes with woven 100% cotton cloth impregnated with pure oil (Aprehend oil without conidia) acted as a control for the oil formulation (oil-control).

2.3.3 *Mortality registration and egg deposition*

Mortality was recorded daily by blowing gently through a mesh opening in the lid of the experimental boxes and observing the odor release behavior. Bed bugs waving their antennae, shifting their stance, or moving were considered alive. Exposure was terminated after 10 days, and any survivors were transferred to a fresh box and evaluated after an additional 7 and 14 days. Dead bed bugs were removed from the boxes and incubated to check for mycosis. The carcasses were individually dried with silica gel before incubation in moist chambers for examination of fungal colonization and sporulation.[37] If fungal hyphae were observed after drying for 4–5 days and rehydration for 7 days, the bed bugs were considered to have died due to fungal infection. Although substantial mortality had occurred, the inoculated substrates covering the bottom of the exposure boxes had deposited eggs attached to the surface. These eggs offered a chance to

investigate any effects on the deposited eggs. Instead of using a transplant experiment, where handling is likely to inflict substantial egg mortality, these substrates were kept for 14 days to check the hatching rate.

2.4 Experiments

Each experimental treatment utilized 48 adult bed bugs evenly distributed across eight boxes (six individuals × eight boxes per treatment), with a balanced sex ratio of three females and three males in each box. The separation of the experimental cohorts into smaller units was done to ease counting and registration of survival. Both fungal products were tested in parallel across all treatment variations, and the balanced male–female experimental design allowed for an overall comparison of gender differences across both products. Except for experiment 1 (dose dependency), the manufacturers' recommended user dosages were utilized and the products were prepared following label instructions (2.0% oil, 0.02% water).

Except for the experiment investigating the effect of elapsed time since feeding, all bed bugs were fed 2 days before the exposure and only fully engorged individuals were included in the experiments. Bed bugs typically engage in mating when females are engorged (days 1 and 2) before they deposit most of their eggs the following week.[38–42] They have depleted most of their resources and are ready to search for a new blood meal after 8–9 days.[43] We used 2 days as the baseline measure and three subsequent 2-day steps to investigate the gradient between engorged bed bugs and bed bugs with more limited water and energy resources.

2.4.1 *Experiment 1: Dose dependency*

To describe the effect of dosages and to compare the water- and oil-based formulations, the two products were prepared to obtain 0.02, 0.125, 0.50, 1.0, or 2.0% conidial concentrations. These concentrations represent a gradient from the recommended dose of the water-based product (0.02%) to the recommended dose for the oil-based product (2.0%). Adult bed bugs were fed and rested before being transferred to the experimental boxes containing woven cotton cloth which had been inoculated with conidia by dipping them in the suspension (five concentrations in water, five concentrations in oil).

2.4.2 *Experiment 2: Application on different substrates*

Several surface materials were evaluated to test field-relevant substrates and applications. These included circular pieces (diameter = 47 mm) of filter paper (Whatman qualitative filter paper; WWR, Oslo, Norway), curtain cloth made from polyester (Lystett gardin grå; Gardinspesialisten, Oslo, Norway), 1 mm thick balsa wood (Balsaflak; Norwegian Modelers, Oslo, Norway), and 2 mm thick linoleum (Grått linoleumsgulv; Statsbygg, Oslo, Norway), which were used as representative materials commonly found in man-made bed bug habitats. Conidia were applied using three sprays with the handheld sprayer on both sides of the substrate. Additionally, the cotton cloth which was used in the dose–response experiment was inoculated with conidia three times, with each inoculation occurring 2 days apart. Repeated applications have not been investigated previously even though application can be performed at inspections, during initial treatment, and on follow-up visits during bed bug control. Adult bed bugs were fed and rested before being transferred into the experimental boxes with the various substrates inoculated with conidia.

2.4.3 *Experiment 3: Aging and reproduction*

Either unfed adult bed bugs kept together in one box for 6 weeks (aged but not reproducing) or bed bugs kept together in one box and fed once every 2 weeks for 6 weeks (aged and reproducing) were used to measure the impact of age and reproductive activity on the efficacy of the fungi. Bed bugs in these two groups were then fed and rested before being transferred to the experimental boxes with cotton woven cloth inoculated with conidia by dipping the substrate in the suspension.

2.4.4 *Experiment 4: Time since feeding*

To investigate potential influences resulting from various times since the last feeding, adult bed bugs were fed and rested for 2, 4, 6, or 8 days before being transferred to the experimental boxes with woven cotton cloth inoculated with conidia by dipping the substrate in the suspension.

2.5 Statistical analysis

Data were analyzed using SigmaPlot 13.0 (Systat Software Inc., San Jose, CA, USA) and JMP pro 13.0.0 (SAS Institute, Cary, NC, USA) software. To investigate the population mortality during the experiments, we conducted survival analysis using the Kaplan–Meier product limit method with the log-rank test. The Kaplan–Meier survival analysis followed predefined populations through time and investigated the fate/survival of individual bed bugs in specific cohorts. Each experiment was conducted twice to separate the testing in time. This ensures that the results are not influenced by potential variations in application method, dilution, environmental conditions, bed bug health, and so forth. Averages were compared using ANOVA. The level of significance was set to 0.05 for all analyses.

3 RESULTS

No mortality was observed among the control insects during the study, while the two fungal products caused 37–100% mortality within 24 days across all experiments. All dead bed bugs showed

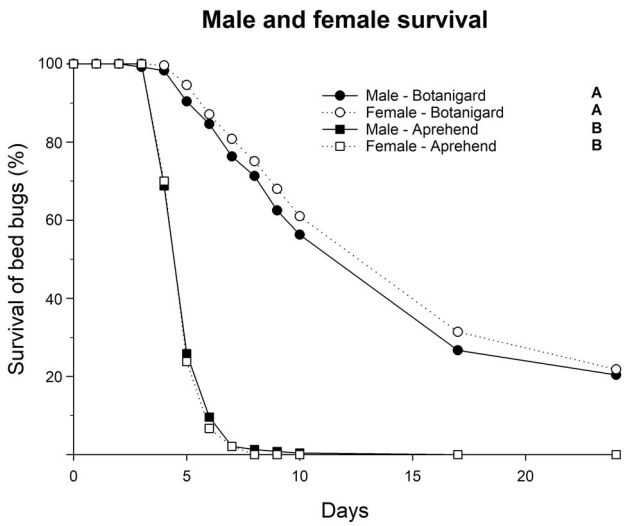

Figure 1. Survival of male and female *Cimex lectularius* after exposure to conidia of *Beauveria bassiana* formulated in water and oil at 0.02% and 2.0%, respectively. Different capital letters (A, B) indicate significant differences in survival between the treatments (Kaplan–Meier survival analysis, all pairwise comparisons, $P < 0.05$). No mortality occurred in the control ($n = 228$ bed bugs).

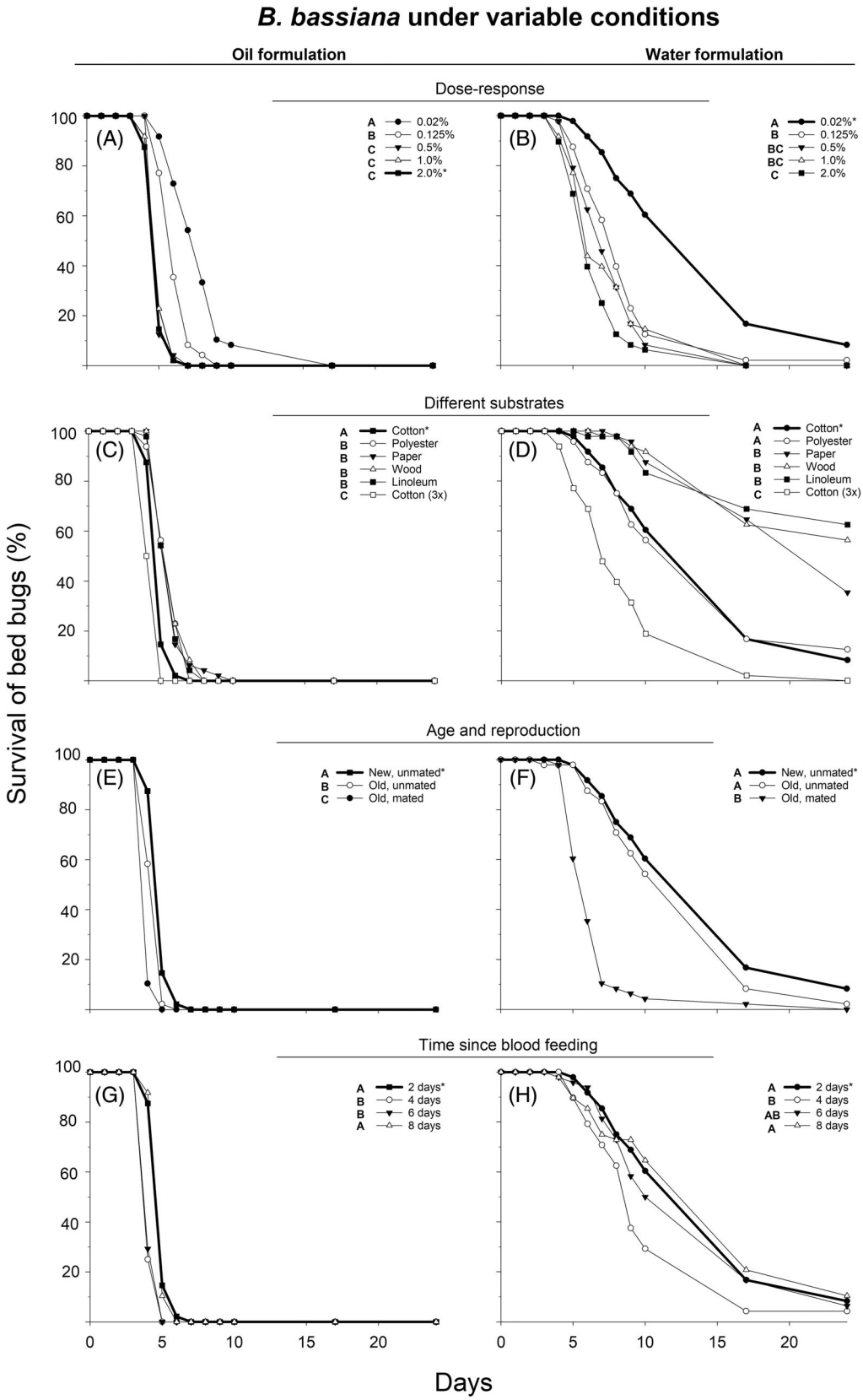

Figure 2. Survival of *Cimex lectularius* after exposure to conidia of *Beauveria bassiana* formulated in water and oil. The bold line in the dose–response experiments (A, B) represents the recommended dosage on the labels of the respective products. This treatment is presented in all graphs as a reference to show the relative effects of different substrates (C, D), age and reproduction (E, F), and time since blood feeding (G, H). Different capital letters A–C indicate significant differences in survival between the treatments (Kaplan–Meier survival analysis, all pairwise comparisons, $P < 0.05$). No mortality occurred in the control ($n = 228$ bed bugs).

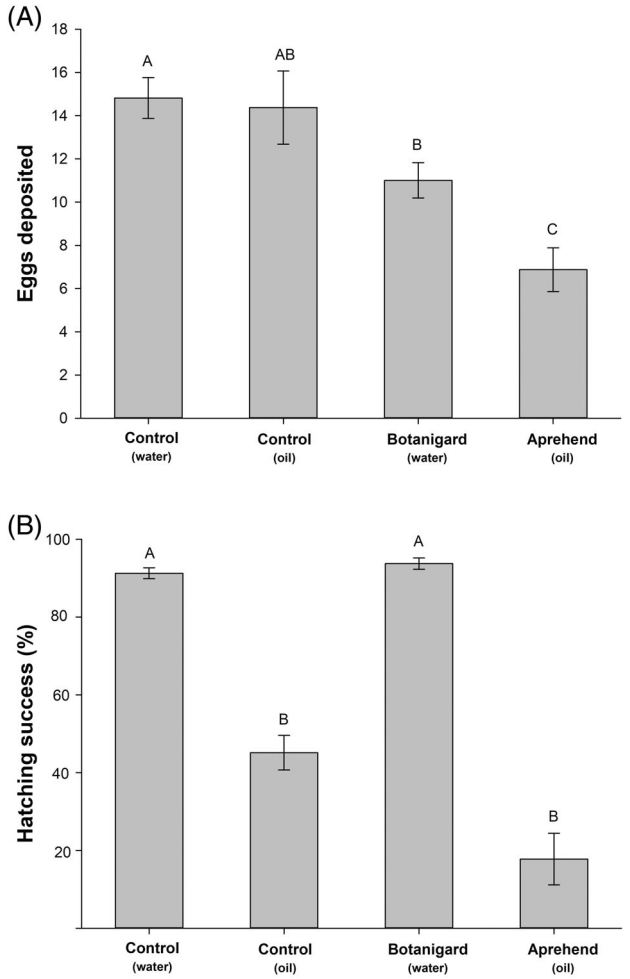

Figure 3. Average number of eggs deposited by three *Cimex lectularius* females (A) and their hatching success (B) on substrates treated with conidia of *Beauveria bassiana* formulated in water or oil compared to untreated control and pure oil control. Different capital letters A, B and C indicate significant differences between the treatments (ANOVA with all pairwise multiple comparison procedures, Dunn's and Holm-Sidak method, $P < 0.05$).

fungal growth when checked for mycosis. Generally, bed bugs exposed to the high-dose oil formulation died faster than those exposed to the low-dose water formulation. Additionally, complete population mortality only occurred in bed bugs exposed to the high-dose oil formulation. Across treatments administered following the labeled dosage, no significant gender differences were observed when using either oil (Kaplan–Meier log-rank test, $\chi^2 = 0.43$, $P = 0.511$) or water (Kaplan–Meier log-rank test, $\chi^2 = 0.94$, $P = 0.333$; Fig. 1).

Both products showed significant dose dependency, with a stepwise change from 0.02% to 2% conidial concentration, significantly affecting survival in both oil (Kaplan–Meier log-rank test, $\chi^2 = 132.01$, $P < 0.001$; Fig. 2(A)) and water (Kaplan–Meier log-rank test, $\chi^2 = 62.67$, $P < 0.001$; Fig. 2(B)). Dosages ≥0.5% produced complete mortality regardless of the product, while doses below this level either failed to achieve full eradication or experienced delayed eradication (all pairwise comparisons are given in Fig. 2(A),(B)).

Application on different substrates significantly affected survival in both oil (Kaplan–Meier log-rank test, $\chi^2 = 113.32$, $P < 0.001$; Fig. 2(C)) and water (Kaplan–Meier log-rank test, $\chi^2 = 158.70$, $P < 0.001$; Fig. 2(D)). Reduced mortality was observed on hard surfaces (paper, wood, and linoleum), and a greatly increased mortality was observed when the products were applied three times. Oil-based product used on polyester was an exception to this pattern by showing reduced mortality on this soft surface (all pairwise comparisons are given in Fig. 2(C),(D)).

Both aging (Kaplan–Meier log-rank test, $\chi^2 = 13.12$, $P < 0.001$) and reproduction (Kaplan–Meier log-rank test, $\chi^2 = 55.78$, $P < 0.001$; Fig. 2(E)) significantly increased mortality when using the oil formulation, whereas with the water formulation only reproduction made a significant difference (Kaplan–Meier log-rank test, $\chi^2 = 55.13$, $P < 0.001$; Fig. 2(F)).

Mortality varied significantly with time since feeding when using both oil (Kaplan–Meier log-rank test, $\chi^2 = 13.20$, $P = 0.004$) and water (Kaplan–Meier log-rank test, $\chi^2 = 12.97$, $P = 0.005$). Exposure on day 4 after feeding induced higher mortality compared to exposure on days 2 and 8 with both products. Exposure on day 6 after feeding also induced a significant higher mortality with oil, whereas water showed intermediate mortality on day 6 (all pairwise comparisons are given in Fig. 2(G),(H)).

Egg production was related to the adult mortality and was significantly reduced by both treatments (analysis of variance (ANOVA) $F = 11.40$, df = 3, $P < 0.001$; Fig. 3(A)). The pairwise comparison showed that egg production with *B. bassiana* formulated in oil was lower than with all other treatments, while *B. bassiana* formulated in water was only lower than the control (water). Hatching success was also reduced significantly (Kruskal–Wallis ANOVA $H = 43.53$, df = 3, $P < 0.001$; Fig. 3(B)). The reduced hatching success was observed in the oil-treatment group as well as in the oil-control group.

4 DISCUSSION

This study confirms previous findings which show that *B. bassiana* is an efficient lethal agent against bed bugs.[6–9] Other fungi, such as *M. anisopliae*, *C. fumosorosea*, and *A. muscarius*, have not shown the same promise at labeled doses or at the relative humidity levels expected to be found in bedrooms.[8,10] The mortality rates for both *Beauveria* products showed a strong dose dependency, and the experiments indicated that the oil-based formulation maintained its killing efficiency at reduced dosages and inflicted more rapid mortality compared to the water-based formulation. The differences can be partly explained by a higher germination percentage (64 *vs* 78%) but are most likely to be a result of the oil formulation. Oil formulations may have improved the durability or infectiousness of the conidia through various means, including greater persistence in the environment or through improved attachment, adhesion, transport to intersegmental folds, germination, or hyphal penetration.[17] However, the use of dry conidia without moisture supply also shows high infection rates against bed bugs.[9] The variations in mortality related to bed bug physiology and substrate were also observed to be more distinct than in the high-dosage treatments when using water-based formulations at low dosages. These low-dosage differences are particularly interesting because they may reflect the more likely limited conidial exposure which would be expected to occur in field applications.

There is a lack of scientific field studies that show the functionality of fungi used against real bed bug infestations, and some laboratory studies even indicate a limited effect due to dry conditions in bedrooms and a potential antifungal property of the

bed bug aggregation chemicals.[10,15] In this respect, *B. bassiana* appears to be a promising agent as mortality occurs with all formulations (water, oil, and dry conidia) and horizontal transfer occurs naturally between individuals in their aggregations.[6–9] Conidial distribution and the resulting bed bug mortality may therefore be effectively optimized by strategic application of fungi-based products. The results of this study follow previous investigations which have also shown that harder surfaces, for example paper with fewer contours, provide suboptimal pick-up of conidia.[7] This study adds knowledge about other relevant substrates and indicates that efficiency and efficacy may be negatively affected by suboptimal use. This is an important aspect because bed bug substrate–behavior interactions are likely to occur during field application. We used forced exposure experiments for an extended period, and this does not represent a true field exposure situation. It would also have been beneficial to know the actual conidia count on different substrates in the spray application experiment to identify mechanisms more precisely. However, the effects were largely comparable when using both oil and water as the conidia carrier, even though the time until death and the degree of mortality differed. Because spray application was done identically on all substrates, the experiment shows substrate effects even though the true conidia density was not identified. Germination percentage, fungal strain, solvent used, and interactions with the substrate may all have affected the mortality rates, but the study indicates strong efficacy at conidial concentrations >0.5% for both products regardless of these experimental discrepancies. With the oil formulation applied at the label rate, all bed bugs died within 5–10 days in all treatments. It is also interesting that the mortality caused by *B. bassiana* appears to be increased with repeated treatments with the 0.02% solution. A single 2% application may leave approximately three times as many potential allergens as an identical application of 0.02% conducted three times. This illustrates a potential to improve the effectiveness with fewer conidia and may therefore ensure efficient control with a reduced risk for humans using the treated premises. A triple application of a 2.0% solution may increase the potential allergen concentration in a room with only a minor benefit related to the timing of mortality. Even if *B. bassiana* is unable to grow in humans, the use of conidia indoors may potentially exacerbate allergies or asthma due to the inhalation of allergens in the form of complex proteins.[31,32,34,44] Some fungal biocontrol agents may even be capable of modulating the mammalian immune system, resulting in impaired homeostasis.[33] Indoor use should therefore be initiated cautiously and further dose-application studies should be conducted.

The physiological state (ageing, reproduction, and feeding) of the bed bugs also affected their survival, but the differences within each experiment were minor and mostly influenced the timing of mortality. These effects are therefore of limited relevance for the overall efficacy in a laboratory setting such as in this study, but they may elucidate how insect pathogenic fungi may work against bed bugs under more realistic field conditions. Mortality rates increased with a moderate degree of senescence, and a mixed-age structure was always observed in established field populations. Death after exposure to a mortal dose of conidia may therefore occur even faster in adult bed bugs found in natural field populations. This is because adults in natural populations are more likely to be reproducing and older than most of the individuals investigated in this study. Only adult insects were used in the experiments in the current study because all instars are similarly susceptible to *B. bassiana*.[7] Juveniles in field populations are therefore expected to have a comparable mortality rate to that observed in the adults in this study, but differences may exist in their ability to pick up spores on different substrates. Future studies of the survival rates among various stages at low dosages should also be conducted to identify potential differences in susceptibility. Increased data regarding nymphs' ability to avoid infection when molting occurs shortly after exposure will be particularly interesting.

Mating often takes place while bed bugs are still engorged, and eggs are typically deposited during the first 4–7 days after feeding.[38–42] The increased mortality rate observed in this study among bed bugs exposed to fungi 4–6 days after feeding may therefore be connected to resource allocation or behavioral changes during such reproductive activities.[45–48] Reproduction takes place continuously in field populations and may consequently ensure high control efficiency if conidia are applied in or close to bed bug harborages. Additionally, thigmotaxis and aggregational behavior may promote horizontal transfer to ensure mortality among unexposed individuals.[7,8,49–51] All these aspects point in the direction of a reasonably high field efficacy, but antifungal properties of the aggregation chemicals may reduce the overall efficacy.[15,16] The ability of bed bugs to disperse away from current aggregations and densely populated parts of an apartment may also allow them to escape from treated areas, resulting in reduced control success.[52]

A crucial control aspect related to decimation efficiency is potential long-term population suppression. Most bedrooms are clean and sheltered environments where the conidia should last for several weeks because many degrading factors are removed.[4,17] A treatment with conidia may therefore kill bed bug offspring if they also encounter the treated area, but a high reproductive output during or after the control efforts has the potential to lessen the treatment's long-term results. Egg production itself was observed to be affected by *B. bassiana* treatment, but this effect is closely linked to the mortality which took place in the initial days of the experiments during oogenesis and egg deposition.[38–40] The oil-based product was observed to act quickly and therefore inflicts the strongest reproductive limitations on the experimental populations. The next, and probably most interesting observation related to long-term effects was the strongly reduced hatching success of the eggs deposited on a surface treated with oil. Egg survival, that is, hatching success, was more than halved in both the oil-based treatment and the oil-control groups. The effect can therefore be assigned to the oil itself. Pure oil has been shown to inflict mortality in bed bug control treatments after both topical application and contact exposure.[9] This aspect will require further study with much better control of the oil content and the surface dryness before and during egg deposition, but the egg mortality in the experiment in this study indicates a potential contribution during control. Eggs are often deposited in hidden locations in small cracks and crevices where the application of a biopesticide is not feasible. This may strongly limit this potential contribution, and practical aspects, for example drying time, oiliness, staining, or odors from oil-based products, may negate any potential benefits.

From an efficiency perspective, product application should be directed toward bed bug aggregations to limit the amount of biopesticide used and to maximize contact probability and subsequent mortality. Product application near bed bug aggregations, typically in human resting places, for example beds, sofas, and chairs, will also increase the risk of human exposure to conidia.[11] Application in these locations also makes removal of the conidia

impractical and more difficult, and may result in chronic exposure to allergens after successful eradication.[32,34] Prioritization of different treatment areas and application strategies will require further study to decipher the interactions between concentration, solvents, and substrates relative to the infectious ability and the persistence of conidia in the environment. The current study has highlighted some aspects that may affect efficiency, efficacy, and application strategies, but it will be crucial to identify the optimum balance between effectiveness and safety, as for many traditional pesticides used indoors.

5 CONCLUSION

The potential benefits and problems associated with bed bug behavior highlight the need for an improved understanding of when, where, and how to use *B. bassiana* as a tool in an IPM strategy. *B. bassiana* appears to be an asset in the fight against bed bugs because it can be used in a manner comparable to traditional pesticides. Insect pathogenic fungi may contribute significantly to bed bug population decimation with the proper dosage and efficient application. The current study is cautiously hopeful for the success of this method, but field studies confirming its functionality in infested apartments remain to be conducted. Future studies which will investigate the contribution of insect pathogenic fungi in real-world bed bug control situations are awaited to truly verify the potential of this method.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

1 de Faria MR and Wraight SP, Mycoinsecticides and mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. *Biol Control* **43**:237–256 (2007).
2 Arthurs S and Dara SK, Microbial biopesticides for invertebrate pests and their markets in the United States. *J Invertebr Pathol* **165**:13–21 (2019).
3 Hajek AE and St Leger RJ, Interactions between fungal pathogens and insect hosts. *Annu Rev Entomol* **39**:293–322 (1994).
4 Jaronski ST, Ecological factors in the inundative use of fungal entomopathogens. *BioControl* **55**:159–185 (2010).
5 Hajek AE, Natural Enemies, in *An Introduction to Biological Control*. Cambridge University Press, New York (2004).
6 Barbarin AM, Bellicanta GS, Osborne JA, Schal C and Jenkins NE, Susceptibility of insecticide-resistant bed bugs (*Cimex lectularius*) to infection by fungal biopesticide. *Pest Manag Sci* **73**:1568–1573 (2017).
7 Barbarin AM, Jenkins NE, Rajotte EG and Thomas MB, A preliminary evaluation of the potential of *Beauveria bassiana* for bed bug control. *J Invertebr Pathol* **111**:82–85 (2012).
8 Aak A, Hage M and Rukke BA, Insect pathogenic fungi and bed bugs: behaviour, horizontal transfer and the potential contribution to IPM solutions. *J Pest Sci* **91**:823–835 (2018).
9 Ashbrook AR, Mikaelyan A and Schal C, Comparative efficacy of a fungal entomopathogen with a broad host range against two human-associated pests. *Insects* **13**:774 (2022).
10 Ulrich KR, Feldlaufer MF, Kramer M and St. Leger RJ, Exposure of bed bugs to *Metarhizium anisopliae* at different humidities. *J Econ Entomol* **107**:2190–2195 (2014).
11 Doggett SL, Miller DM and Lee CY, *Advances in the Biology and Management of Modern Bed Bugs*. John Wiley & Sons Ltd, Oxford, UK (2018).
12 Pietri JE and Liang DS, Virulence of entomopathogenic bacteria in the bed bug, *Cimex lectularius*. *J Invertebr Pathol* **151**:1–6 (2018).
13 Shikano I, Bellicanta GS, Principato S and Jenkins NE, Effects of chemical insecticide residues and household surface type on a *Beauveria bassiana*-based biopesticide (Aprehend®) for bed bug management. *Insects* **12**:214 (2021).
14 Shikano I, Efficacy of a fungal biopesticide for bed bug management is influenced by the toxicity and associated behavioral avoidance of harborages on insecticide-impregnated box spring covers. *J Econ Entomol* **113**:2850–2857 (2020).
15 Ulrich KR, Feldlaufer MF, Kramer M and St Leger RJ, Inhibition of the entomopathogenic fungus *Metarhizium anisopliae* sensu lato in vitro by the bed bug defensive secretions (E)-2-hexenal and (E)-2-octenal. *Biocontrol* **60**:517–526 (2015).
16 Dery M and Choe D-H, Effect of bed bug (Hemiptera: Cimicidae) aldehydes on efficacy of fungal biopesticides. *J Econ Entomol* **116**:40–46 (2022).
17 Jackson MA, Dunlap CA and Jaronski ST, Ecological considerations in producing and formulating fungal entomopathogens for use in insect biocontrol. *Biocontrol* **55**:129–145 (2010).
18 Acharya N, Rajotte EG, Jenkins NE and Thomas MB, Influence of biotic and abiotic factors on the persistence of a *Beauveria bassiana* biopesticide in laboratory and high-rise poultry house settings. *Biocontrol Sci Technol* **25**:1317–1332 (2015).
19 Gindin G, Samish M, Zangi G, Mishoutchenko A and Glazer I, The susceptibility of different species and stages of ticks to entomopathogenic fungi. *Exp Appl Acarol* **28**:283–288 (2002).
20 Kirkland BH, Cho EM and Keyhani NO, Differential susceptibility of *Amblyomma maculatum* and *Amblyomma americanum* (Acari:Ixodidea) to the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae*. *Biol Control* **31**:414–421 (2004).
21 Rukke BA, Salma U, Birkemoe T and Aak A, Blood deprivation and heat stress increase mortality in bed bugs (*Cimex lectularius*) exposed to insect pathogenic fungi or desiccant dust. *Med Vet Entomol* **35**:121–128 (2021).
22 Dang K, Doggett SL, Veera Singham G and Lee CY, Insecticide resistance and resistance mechanisms in bed bugs, *Cimex* spp. (Hemiptera: Cimicidae). *Parasit Vectors* **10**:318 (2017). https://doi.org/10.1186/s13071-13017-12232-13073.
23 Rukke BA, Roligheten E and Aak A, Procurement competence and framework agreements for upgraded bed bug control [*Cimex lectularius* (Hemiptera: Cimicidae)]. *J Econ Entomol* **115**:240–249 (2022).
24 Doggett SL, Miller DM, Vail K and Wilson MS, Fiscal impacts, in *Advances in the Biology and Management of Modern Bed Bugs*, ed. by Doggett SL, Miller DM and Lee CY. John Wiley & Sons Ltd: Oxford, UK, pp. 139–148 (2018).
25 Doggett SL and Lee C-Y, Historical and contemporary control options against bed bugs, *Cimex* spp. *Annu Rev Entomol* **68**:169–190 (2023).
26 Romero A, Sutherland AM, Gouge DH, Spafford H, Nair S, Lewis V *et al*., Pest management strategies for bed bugs (Hemiptera: Cimicidae) in multiunit housing: a literature review on field studies. *J Integr Pest Manag* **8**:1–10 (2017).
27 Bennett GW, Gondhalekar AD, Wang C, Buczkowski G and Gibb TJ, Using research and education to implement practical bed bug control programs in multifamily housing. *Pest Manag Sci* **72**:8–14 (2016).
28 Koganemaru R and Miller DM, The bed bug problem: past, present, and future control methods. *Pestic Biochem Physiol* **106**:177–189 (2013).
29 Rani L, Thapa K, Kanojia N, Sharma N, Singh S, Grewal AS *et al*., An extensive review on the consequences of chemical pesticides on human health and environment. *J Clean Prod* **283**:124657 (2021).

30 Mallis A, Hedges SA and Moreland D, *Handbook of Pest Control: The Behaviour, Life History, and Control of Household Pests*. Mallis Handbook & Technical Training Company, USA (2011).
31 Kanchongkittiphon W, Mendell MJ, Gaffin JM, Wang G and Phipatanakul W, Indoor environmental exposures and exacerbation of asthma: an update to the 2000 review by the Institute of Medicine. *Environ Health Perspect* **123**:6–20 (2015).
32 Madsen AM, Hansen VM, Meyling NV and Eilenberg J, Human exposure to airborne fungi from genera used as biocontrol agents in plant production. *Ann Agric Environ Med* **14**:5–24 (2007).
33 Konstantinovas C, de Oliveira Mendes TA, Vannier-Santos MA and Lima-Santos J, Modulation of human immune response by fungal biocontrol agents. *Front Microbiol* **8**:39 (2017). https://doi.org/10.3389/fmicb.2017.00039.
34 Madsen AM, Occupational exposure to microorganisms used as biocontrol agents in plant production. *Front Biosci* **3**:606–620 (2011).
35 Aak A and Rukke BA, Bed bugs, their blood sources and life history parameters: a comparison of artificial and natural feeding. *Med Vet Entomol* **28**:50–59 (2014).
36 Oliveira DGP, Pauli G, Mascarin GM and Delalibera I, A protocol for determination of conidial viability of the fungal entomopathogens *Beauveria bassiana* and *Metarhizium anisopliae* from commercial products. *J Microbiol Methods* **119**:44–52 (2015).
37 Butt TM and Goettel MS, Bioassays of entomogenous fungi, in *Bioassays of Entomopathogenic Microbes and Nematodes*, ed. by Navon A and Ascher KRS. Wallingford, CABI Publishing, pp. 141–195 (2000).editors.
38 Matos YK, Osborne JA and Schal C, Effects of cyclic feeding and starvation, mating, and sperm condition on egg production and fertility in the common bed bug (Hemiptera: Cimicidae). *J Med Entomol* **54**: 1483–1490 (2017).
39 Saveer AM, DeVries ZC, Santangelo RG and Schal C, Mating and starvation modulate feeding and host-seeking responses in female bed bugs, *Cimex lectularius*. *Sci Rep* **11**:1915 (2021).
40 Polanco AM, Miller DM and Brewster CC, Reproductive potential of field-collected populations of *Cimex lectularius* L. and the cost of traumatic insemination. *Insects* **2**:326–335 (2011).
41 Reinhardt K, Naylor RA and Siva-Jothy MT, Situation exploitation: higher male mating success when female resistance is reduced by feeding. *Evolution* **63**:29–39 (2009).
42 Gershman SN, Harrison SA and Jones SC, Opportunities for female choice in the bed bug *Cimex lectularius*. *Ethology* **125**:92–97 (2019).
43 Aak A, Rukke BA, Soleng A and Rosnes MK, Questing activity in bed bug populations: male and female responses to host signals. *Physiol Entomol* **39**:199–207 (2014).
44 Zimmermann G, Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. *Biocontrol Sci Technol* **17**:553–596 (2007).
45 Reinhardt K, Naylor RA and Siva-Jothy MT, Potential sexual transmission of environmental microbes in a traumatically inseminating insect. *Ecol Entomol* **30**:607–611 (2005).
46 Benoit JB, Jajack AJ and Yoder JA, Multiple traumatic insemination events reduce the ability of bed bug females to maintain water balance. *J Comp Physiol B* **182**:189–198 (2012).
47 Siva-Jothy MT, Zhong WH, Naylor R, Heaton L, Hentley W and Harney E, Female bed bugs (*Cimex lectularius* L) anticipate the immunological consequences of traumatic insemination via feeding cues. *Proc Natl Acad Sci USA* **116**:14682–14687 (2019).
48 Bellinvia S, Spachtholz A, Borgwardt I, Schauer B and Otti O, Female immunity in response to sexually transmitted opportunistic bacteria in the common bedbug *Cimex lectularius*. *J Insect Physiol* **123**:104048 (2020).
49 Akhtar Y and Isman MB, Horizontal transfer of diatomaceous earth and botanical insecticides in the common bed bug, *Cimex lectularius* L.; Hemiptera: Cimicidae. *PLoS ONE* **8**:e75626 (2013).
50 Gries R, Britton R, Holmes M, Zhai H, Draper J and Gries G, Bed bug aggregation pheromone finally identified. *Angew Chem Int Ed* **54**: 1135–1138 (2015).
51 Siljander E, Penman D, Harlan H and Gries G, Evidence for male- and juvenile-specific contact pheromones of the common bed bug *Cimex lectularius*. *Entomol Exp Appl* **125**:215–219 (2007).
52 Cooper R, Wang C and Singh N, Mark-release-recapture reveals extensive movement of bed bugs (*Cimex lectularius* L.) within and between apartments. *PLoS ONE* **10**:e0136462 (2015).

