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Synergistic Botanical Spatial Repellency and Entomopathogenic Fungal Domino Pathogenicity in a Novel Pea-Fiber Feline Litter Matrix

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Research Article

Keywords: pea fiber, feline litter, botanical repellent, spatial repellency, entomopathogenic fungi, Beauveria bassiana, insect pest control, synergistic effect

Posted Date: November 18th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11763481/v1

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Additional Declarations: The authors declare potential competing interests as follows: The authors are inventors of the pea-fiber litter formulation described in this preprint and have a pending patent / commercial interest in its development.

Synergistic Botanical Spatial Repellency and Entomopathogenic Fungal Domino Pathogenicity in a Novel Pea-Fiber Feline Litter Matrix Against Pyrethroid-Resistant *Cimex lectularius* and Companion Animal Ectoparasites

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Technical Study Reference: GCC-R&D-2026-CL01 / EXP01

Abstract

Domestic pest management in cat-owning households is severely constrained by the evolutionary pseudogenization of feline hepatic UDP-glucuronosyltransferase (*UGT1A6*), which predisposes cats (*Felis catus*) to neurotoxicity and hepatotoxicity upon exposure to synthetic pyrethroids and concentrated botanical essential oils. Concurrently, the global resurgence of pyrethroid-resistant common bed bugs (*Cimex lectularius*) alongside persistent ectoparasites such as cat fleas (*Ctenocephalides felis*) and brown dog ticks (*Rhipicephalus sanguineus*) necessitates non-chemical intervention paradigms. Because standardized commercial testing protocols do not yet exist for solid granular feline litter substrates incorporating biological control agents, an integrated multi-stage bioassay framework was developed to evaluate a novel dual-action pea-fiber tofu cat litter matrix (95.0% food-grade pea fiber and soybean dregs, 4.5% essential-oil-free steam-distilled *Nepeta cataria* hydrosol, and 0.5% dormant *Beauveria bassiana* [*G. Bassiana*] conidia at $\geq 1 \times 10^8$ CFU/g). In two-choice glass Y-tube olfactometer assays ($n = 3$ triplicates), the hydrated matrix yielded potent gas-phase spatial repellency indices (RI) of $+80.0$–82.6% against litter-breeding dipterans ($\chi^2 \approx 28.9, p < 0.001$), $+85.0$–86.0% against hematophagous mosquitoes ($\chi^2 \approx 33.4, p < 0.001$), $+72.7$–75.0% against *C. felis* ($\chi^2 \approx 21.8, p < 0.001$), and $+68.8$–71.0% against *C. lectularius* ($\chi^2 \approx 19.3, p < 0.001$). In forced-contact and transient-exposure bioassays, Abbott's corrected mortality at Day 7 reached 98.0–100% ($LT_{50} \approx 2.3$ d) in *C. lectularius* nymphs, 96.5–99.0% ($LT_{50} \approx 2.6$ d) in *C. lectularius* adults, 98.5–100% ($LT_{50} \approx 2.1$ d) in *C. felis*, and 94.0–96.5% ($LT_{50} \approx 2.9$ d) in *R. sanguineus*, with 88.0–95.0% cadaver mycosis confirmation. Furthermore, a 1:5 donor-to-recipient crevice harborage assay demonstrated 84.0–90.0% secondary mortality in unexposed bed bug colonies ($LT_{50} \approx 3.8$ d). Behavioral monitoring across five domestic cats confirmed 100% litter box acceptance with zero salivation, neurological hyper-excitation, or periuria. These findings establish solid-matrix botanical–mycoinsecticidal synergy as an effective, mammalian-safe passive household defense strategy.

Keywords: *Beauveria bassiana*; *Nepeta cataria* hydrosol; *Cimex lectularius*; *Ctenocephalides felis*; horizontal conidial transfer; Y-tube olfactometer; feline hepatic safety; biological pest control.

1 Introduction

Companion animal households represent complex ecological interfaces where hematophagous ectoparasites and synanthropic nuisance insects converge. Cat fleas (*Ctenocephalides felis*),

brown dog ticks (*Rhipicephalus sanguineus*), litter-colonizing dipterans (phorid flies, family Phoridae, and drosophilid flies), and resurgent populations of the common bed bug (*Cimex lectularius*) frequently exploit micro-habitats surrounding pet sleeping and elimination zones. Historically, indoor suppression of these arthropods has relied upon residual synthetic insecticides, predominantly pyrethroids and neonicotinoids. However, deploying synthetic neurotoxins or concentrated botanical essential oils in feline environments poses severe toxicological hazards. Domestic cats and other hypercarnivorous felids carry fixed disruptive mutations rendering the hepatic UDP-glucuronosyltransferase gene *UGT1A6* a non-functional pseudogene [4]. Deficient glucuronidation capacity markedly impairs feline clearance of phenolic compounds, monoterpenes, and pyrethroid esters, leading to acute neurotoxicity or cumulative hepatic injury following dermal paw contact and oral grooming.

Simultaneously, chemical pest control efficacy against indoor hematophagous pests has eroded due to widespread insecticide resistance. Field-collected populations of *C. lectularius* across dense urban centers exhibit high-frequency knockdown resistance (*kdr*) point mutations in voltage-gated sodium channels, upregulated cytochrome P450 monooxygenases, and cuticular thickening that dramatically reduce susceptibility to pyrethroid sprays [3]. Consequently, urban multi-pet households require alternative integrated pest management (IPM) modalities that bypass chemical target-site resistance while maintaining an uncompromising toxicological safety profile for cats.

A promising biological approach involves pairing selective botanical spatial repellents with entomopathogenic fungi. Nepetalactone, an iridoid monoterpene present in catnip (*Nepeta cataria*), elicits broad-spectrum avoidance across dipterans and other arthropods by selectively activating insect isoforms of the nociceptive transient receptor potential ankyrin 1 (TRPA1) ion channel without activating mammalian TRPA1 [5]. When recovered as an aqueous steam-distilled hydrosol rather than a concentrated lipophilic essential oil, trace water-soluble nepetalactone isomers can establish a mild gas-phase repellent plume around the litter tray without triggering feline hepatic stress or behavioral overstimulation. For pests that breach the perimeter or transiently traverse the substrate, the entomopathogenic ascomycete *Beauveria bassiana* provides a non-chemical lethal mechanism. Lipophilic conidia adhere to the arthropod epicuticle, germinate, and breach the exoskeleton through mechanical turgor and enzymatic degradation, killing pyrethroid-susceptible and pyrethroid-resistant *C. lectularius* alike while spreading horizontally among gregarious nestmates [2, 3].

Despite these complementary mechanisms, embedding viable fungal conidia and aqueous botanical hydrosols into a solid clumping cat litter pellet presents a unique formulation and evaluation challenge. Commercial testing laboratories currently lack standardized operating procedures (SOPs) to evaluate solid hygroscopic litter granules combining gas-phase repellency with contact mycoinsecticidal transfer. Adapting World Health Organization (WHO) and U.S. Environmental Protection Agency (EPA) biological control evaluation principles, this study designed and executed a four-stage laboratory bioassay and clinical tolerance framework to quantify: (1) gas-phase spatial repellency in a two-choice Y-tube olfactometer, (2) forced-contact and transient-contact mortality kinetics across resistant bed bugs, fleas, and ticks, (3) horizontal conidial autodissemination within simulated harborages, and (4) feline behavioral acceptance in domestic settings.

2 Materials and Methods

2.1 Formulation Architecture and Control Substrates

The experimental formulation, designated GCC Professional Dual-Action Pest-Control Tofu Cat Litter (Treatment Group; Green Concept Control, Hong Kong), was engineered as cylindri-

cal extruded granules (1.5–2.0 mm diameter) comprising three functional tiers:

1. **Hygroscopic Physical Matrix (95.0% w/w):** Food-grade pea fiber and refined soybean dregs engineered for rapid liquid clumping, micro-environmental desiccation, and ultra-low respirable dust generation.
2. **First-Line Spatial Repellent Component (4.5% w/w):** Low-concentration aqueous *Nepeta cataria* hydrosol obtained via steam distillation, containing trace dissolved *Z,E*- and *E,Z*-nepetalactone isomers and verified to be strictly free of concentrated lipophilic essential oils.
3. **Second-Line Mycoinsecticidal Component (0.5% w/w):** Patented dormant conidia of *Beauveria bassiana* (designated strain *G. Bassiana*) surface-stabilized onto the exterior pellet matrix at a viable spore titer of $\geq 1 \times 10^8$ CFU/g.

The Blank Control Group consisted of 100.0% pure pea-fiber and soybean-dregs standard tofu cat litter manufactured to identical pellet dimensions (1.5–2.0 mm) without *N. cataria* hydrosol or fungal conidia.

2.2 Target Arthropod Colonies

Four taxonomic arthropod groups were maintained under standardized insectary conditions ($25 \pm 2\,^\circ$C, 65 ± 5% relative humidity [RH]) prior to bioassay allocation:

- **Common Bed Bugs (*Cimex lectularius*):** Cohorts included field-collected pyrethroid-resistant strains stratified into unfed adults and 3rd–5th instar nymphs to evaluate age- and cuticle-dependent susceptibility.
- **Cat Fleas (*Ctenocephalides felis*):** Newly emerged, unfed healthy adult fleas.
- **Brown Dog Ticks (*Rhipicephalus sanguineus*):** Active, unfed adult ticks.
- **Dipteran Flies and Mosquitoes:** Litter-breeding phorid flies (family Phoridae) and fruit flies (*Drosophila melanogaster*), alongside host-seeking female mosquitoes (*Aedes albopictus* and *Culex* spp.).

All experimental evaluations were conducted using a double-blinded randomized design in three independent biological replicates ($n = 3$ triplicates) per cohort.

2.3 Experiment 1: Two-Choice Y-Tube Olfactometer Spatial Repellency Assay

Gas-phase spatial repellency of the 4.5% *N. cataria* hydrosol tier was quantified using a high-borosilicate glass Y-tube olfactometer consisting of a 15 cm central main stem and two 10 cm diverging arms (Treatment Arm A and Control Arm B) set at an inter-arm angle of 75° with an internal diameter of 2.5 cm. Each distal arm terminated in a 250 mL glass odor-source chamber. Incoming air was purified through an activated-charcoal cartridge, humidified via a distilled-water gas-washing bottle, and regulated by precision flowmeters to deliver a symmetric unidirectional laminar flow of 200 mL/min through each arm.

To simulate realistic volatile release upon feline urination, 50.0 g of GCC Treatment Litter (Arm A) and 50.0 g of Blank Control Litter (Arm B) were hydrated immediately prior to testing with 10.0 mL of sterile physiological saline pre-warmed to $38\,^\circ$C. Additionally, supplementary open two-choice arena hydration trials (100 g substrate hydrated with 20 mL warm saline) were monitored up to 4 h to verify sustained repellency. All olfactometer runs were performed

at $25 \pm 1\,^{\circ}$C and 65 ± 5% RH inside a darkroom illuminated exclusively by dim red light (wavelength > 650 nm, outside insect spectral sensitivity) to eliminate phototactic bias. Between replicates, the Y-tube assembly was rotated 180°, washed sequentially with anhydrous ethanol and deionized water, and oven-dried.

For each replicate, $N_{\text{total}} = 50$ healthy test arthropods were released at the main stem entrance. At designated exposure intervals (30 min for mosquitoes; 60 min for phorid/fruit flies, fleas, and bed bugs), individuals crossing > 3 cm past the bifurcation line and remaining for > 1 min were scored as choosing Treatment (N_T) or Control (N_C), while non-choosing individuals in the stem were recorded as N_0 ($N_{\text{total}} = N_T + N_C + N_0$). Four quantitative metrics were computed:

$$PR\ (\%) = \left(\frac{N_C - N_T}{N_C} \right) \times 100\% \tag{1}$$

$$RI\ (\%) = \left(\frac{N_C - N_T}{N_C + N_T} \right) \times 100\% \tag{2}$$

$$RR\ (\%) = \left(\frac{N_C + N_T}{N_{\text{total}}} \right) \times 100\% \tag{3}$$

$$\chi^2 = \frac{(|N_C - N_T| - 1)^2}{N_C + N_T} \quad (df = 1) \tag{4}$$

where PR denotes Percentage Repellency relative to control attraction, RI denotes the bilateral Olfactometer Repellency Index (ranging from -100% attraction to $+100$% repellency, with $RI > +50$% classified as significant and $RI > +75$% as potent spatial repellency), RR denotes the Effective Response Rate (quality-control validity threshold set at $RR \geq 80$%), and χ^2 represents the Yates continuity-corrected Chi-square goodness-of-fit statistic against the $1:1$ null distribution ($H_0 : N_C = N_T$, with $\chi^2 > 3.84$ corresponding to $p < 0.05$ and $\chi^2 > 10.83$ corresponding to $p < 0.001$).

2.4 Experiment 2: Forced-Contact Cuticular Penetration Bioassay

To evaluate direct contact pathogenicity of the 0.5% *B. bassiana* spore tier, sterile 15 cm Petri dishes were layered to a depth of 3 cm with either GCC Treatment Litter or Blank Control Litter ($25 \pm 2\,^{\circ}$C, 65 ± 5% RH). Cohorts of 30 healthy *C. felis* adults or 30 *R. sanguineus* adults per replicate were introduced onto the granular bed and allowed to crawl freely among the pellets for 60 min before transfer to clean, untreated observation dishes. Mortality (defined as complete absence of appendage movement upon gentle mechanical probing) was scored every 24 h over 7 days (168 h). Treatment mortality was normalized for baseline control attrition using Abbott's formula [1]:

$$\text{Corrected Mortality}\ (\%) = \left(\frac{M_T - M_C}{100 - M_C} \right) \times 100\% \tag{5}$$

where M_T and M_C denote observed percentage mortality in the treatment and blank control cohorts, respectively. Median lethal time (LT_{50}) was interpolated from cumulative time–mortality trajectories. All recovered cadavers were surface-sanitized and incubated on moist filter paper at > 90% RH for 5 days to confirm diagnostic white muscardine hyphal emergence across intersegmental membranes (mycosis confirmation).

2.5 Experiments 3 and 4: Horizontal Conidial Transfer and Bed Bug Colony Bioassays

General horizontal autodissemination (Experiment 3) was evaluated by exposing 5 "Donor" insects (marked dorsally with inert fluorescent tracer dust) to GCC Treatment Litter for 30 min and introducing them into a simulated harborage containing 25 unexposed healthy "Recipient" conspecifics (1:5 donor-to-recipient ratio). Secondary mortality among unexposed Recipients was tracked over 7–10 days.

Because *C. lectularius* exhibits pronounced thigmotaxis and spends minimal time outside narrow crevices except during nocturnal host-seeking, Experiment 4 established two specialized bed bug protocols:

1. **Transient Contact Bioassay:** Separate cohorts of pyrethroid-resistant *C. lectularius* adults ($n = 30$ per replicate) and 3rd–5th instar nymphs ($n = 30$ per replicate) traversed a Petri dish bed of GCC Treatment Litter for exactly 15 min (simulating brief nocturnal transit across the litter perimeter or passive drop-off from feline fur), followed by transfer into clean dishes containing accordion-folded filter paper harborages for 7-day mortality and sub-lethal feeding observation.
2. **Harborage Domino Colony Eradication Assay:** Three (3) *C. lectularius* donors exposed to GCC Treatment Litter for 15 min were introduced into corrugated-cardboard crevice models housing 15 unexposed healthy recipients (1:5 ratio). Driven by aggregation pheromones, close cuticular contact within the crevices facilitated conidial exchange, which was quantified via 7-day recipient mortality and cadaver mycosis rates.

2.6 Feline Behavioral Tolerance and In-Home Field Trial Protocol

Clinical behavioral tolerance to the 4.5% *N. cataria* hydrosol litter was evaluated across five domestic cats spanning distinct breeds and life stages (including a juvenile Siamese, an adult Maine Coon, and a senior British Shorthair). Assessed ethological endpoints included sniffing latency, rolling or hyper-excitation inside the tray, pica/granule chewing, salivation, digging and covering compliance, and periuria (inappropriate elimination outside the tray). In parallel, a standardized 30-day multi-cat household field monitoring protocol was established for 10–15 Hong Kong households (≥ 2 cats per home, anchored by a 5-cat benchmark household), combining unscented optical sticky traps positioned adjacent to litter boxes and bed frames (comparing a 7-day pre-transition baseline against Days 14 and 30 post-transition) with owner Visual Analog Scale (VAS, 1–10) assessments of clumping speed, dust suppression, odor control, and feline calmness.

3 Results

3.1 Gas-Phase Spatial Repellency and Statistical Verification

Hydration of GCC Treatment Litter with $38\,^{\circ}$C physiological saline rapidly liberated trace aqueous nepetalactone volatiles without detectable pungent essential-oil odor. Analytical verification is illustrated by a single 60-minute replicate against litter-breeding dipterans ($N_{\text{total}} = 50$), in which $N_C = 42$ flies entered the control arm, $N_T = 4$ entered the GCC treatment arm, and $N_0 = 4$ remained in the stem. Applying Equations (1)–(4) yielded an effective response rate of $RR = (46/50) \times 100\% = 92.0\%$ (exceeding the $\geq 80\%$ quality threshold), a bilateral repellency index of $RI = (38/46) \times 100\% = +82.61\%$, a percentage repellency of $PR = (38/42) \times 100\% = 90.48\%$, and a Yates-corrected Chi-square statistic of $\chi^2 = 37^2/46 = 29.76$ ($p < 0.001$).

Across all four evaluated pest taxa in triplicate Y-tube olfactometer runs, spatial avoidance remained statistically robust ($p < 0.001$; Table 1). Hematophagous mosquitoes exhibited the highest sensitivity ($RI = +85.0$–86.0% within 30 min), followed by phorid and fruit flies ($RI = +80.0$–82.6%), adult cat fleas ($RI = +72.7$–75.0%), and adult bed bugs ($RI = +68.8$–71.0%). Open two-choice arena trials confirmed sustained spatial deterrence up to 4 h post-hydration (72.7–84.2% repellency against *C. felis*).

Table 1: Gas-phase spatial repellency of hydrated GCC Dual-Action Tofu Cat Litter (4.5% *Nepeta cataria* hydrosol) in two-choice Y-tube olfactometer bioassays (mean of triplicates, $n = 3$).

Target Pest Species	**Time**	N_{total}	N_C **Mean**	N_T **Mean**	RI **(%)**	PR **(%)**	χ^2, p**-value**
Phorid / Fruit Flies (*Phoridae* / *D. melanogaster*)	60 min	50	~ 42.3	~ 4.7	$+80.0$–82.6	88.9–90.5	$28.9,\ p < 0.001$
Mosquitoes (*Aedes* / *Culex* spp.)	30 min	50	~ 43.7	~ 3.3	$+85.0$–86.0	92.0–92.4	$33.4,\ p < 0.001$
Cat Fleas (*Ctenocephalides felis*, adults)	60 min	50	~ 38.0	~ 6.0	$+72.7$–75.0	84.2–85.0	$21.8,\ p < 0.001$
Common Bed Bugs (*Cimex lectularius*, adults)	60 min	50	~ 36.3	~ 6.7	$+68.8$–71.0	81.5–83.0	$19.3,\ p < 0.001$

3.2 Direct Contact Pathogenicity, LT_{50} Kinetics, and Mycosis Confirmation

Exposure to the 0.5% *B. bassiana* spore-coated granules produced characteristic sigmoidal entomopathogenic mortality curves across all target ectoparasites (Table 2). During the initial 24–48 h post-exposure window—corresponding to epicuticular conidial adhesion, germ-tube formation, and enzymatic cuticular penetration—mortality remained moderate (6.5–15.2% at Day 1; 22.0–45.0% at Day 2). Between Day 3 (72 h) and Day 5 (120 h), mortality surged exponentially as fungal blastospores proliferated within the hemocoel and secreted insecticidal cyclodepsipeptides (including beauvericin).

Table 2: Cumulative Abbott's corrected mortality (%), median lethal time (LT_{50}), and humid-chamber cadaver mycosis confirmation following contact or horizontal transfer exposure to GCC Dual-Action Tofu Cat Litter ($n = 3$ triplicates).

Target Cohort and Exposure Mode	**D1**	**D2**	**D3**	**D5**	**Day 7 (168 h)**	LT_{50}	**Mycosis Rate and Anatomical Site**
C. lectularius Nymphs (15-min transient)	12.0%	38.5%	76.0%	96.7%	98.0–100%	~ 2.3 d	~ 95.0% (thin molting cuticle)
C. lectularius Adults (15-min transient)	8.5%	28.0%	64.0%	91.5%	96.5–99.0%	~ 2.6 d	~ 92.0% (intersegmental and leg joints)
C. lectularius Domino Colony (1:5 harborage)	0.0%	11.0%	35.5%	72.0%	84.0–90.0%	~ 3.8 d	~ 85.0% (crevice cluster transfer)
C. felis Adults (60-min direct contact)	15.2%	45.0%	68.5%	94.0%	98.5–100%	~ 2.1 d	~ 93.0% (diffuse white mycelium)
R. sanguineus Adults (60-min direct contact)	6.5%	22.0%	52.0%	86.0%	94.0–96.5%	~ 2.9 d	~ 88.0% (spiracles and mouthparts)
General Pest Domino Cohort (1:5 unexposed)	0.0%	8.5%	24.0%	62.5%	78.0–85.0%	~ 4.1 d	> 80.0% (horizontal transmission)
Blank Control (100% pea-fiber tofu litter)	0.0%	1.5%	3.3%	5.0%	6.7%	N/A	0.0% (natural attrition; zero mycosis)

Adult cat fleas exhibited the fastest lethal progression ($LT_{50} \approx 2.1$ d; Day-7 mortality 98.5–100%), closely followed by transient-exposed *C. lectularius* 3rd–5th instar nymphs ($LT_{50} \approx 2.3$ d; Day-7

mortality 98.0–100%) and pyrethroid-resistant *C. lectularius* adults ($LT_{50} \approx 2.6$ d; Day-7 mortality 96.5–99.0%). Despite heavily sclerotized scuta, adult brown dog ticks reached 94.0–96.5% corrected mortality by Day 7 ($LT_{50} \approx 2.9$ d), with fungal outgrowth concentrated around spiracular plates and capitulum articulations. Blank control mortality remained ≤ 6.7% at Day 7 with 0.0% mycosis, confirming that high lethality derived specifically from fungal infection rather than mechanical abrasion alone.

3.3 Horizontal Autodissemination and Sub-Lethal Feeding Inhibition

In the 1:5 crevice harborage domino assay (Experiment 4), introducing just three spore-contaminated *C. lectularius* donors into an unexposed group of 15 healthy bed bugs drove 35.5% corrected mortality by Day 3, 72.0% by Day 5, and 84.0–90.0% by Day 7 ($LT_{50} \approx 3.8$ d), with ~ 85.0% of unexposed recipient cadavers exhibiting confirmed white muscardine mycosis. Similarly, general pest horizontal transfer cohorts (Experiment 3) achieved 78.0–85.0% secondary mortality by Day 7 ($LT_{50} \approx 4.1$ d).

Crucially, during the 24–48 h pre-lethal incubation phase, transient-exposed *C. lectularius* cohorts demonstrated pronounced sub-lethal pathology: blood-feeding propensity declined by > 80% accompanied by progressive locomotor ataxia, while surviving gravid females exhibited sharply curtailed oviposition and egg hatchability prior to death.

3.4 Feline Clinical Behavioral Tolerance

Across all five monitored domestic cats, introduction of the 4.5% *N. cataria* hydrosol tofu litter resulted in 100% voluntary tray utilization. None of the evaluated cats displayed hyperexcitable rolling inside the soiled litter box, compulsive pellet ingestion, or sialorrhea (salivation). Subordinate individuals within multi-cat environments exhibited calm sniffing and normal post-elimination covering behavior, with zero episodes of odor aversion or periuria recorded throughout observation.

4 Discussion

4.1 Physical–Biological Synergy and Circumvention of *kdr* Insecticide Resistance

The high mortality achieved across pyrethroid-resistant *C. lectularius*, *C. felis*, and *R. sanguineus* reflects functional synergy between the hygroscopic pea-fiber substrate and surface-bound *B. bassiana* conidia. Physically, the 95.0% pea-fiber and soybean-dregs matrix rapidly sequesters free moisture upon urination, desiccating the litter micro-environment and disrupting the high-humidity regime (> 70% RH) required for flea larval survival and egg eclosion. Simultaneously, low-dust granular friction facilitates electrostatic transfer of lipophilic *B. bassiana* conidia onto the lipid epicuticle of traversing arthropods.

Unlike synthetic pyrethroids or neonicotinoids that require trans-cuticular diffusion to bind neuronal ion channels, *B. bassiana* differentiates specialized appressoria that secrete extracellular chitinases, subtilisin-like proteases, and lipases to enzymatically digest and mechanically breach the chitin–protein matrix of the exoskeleton [3]. Consistent with prior findings demonstrating uncompromised *B. bassiana* virulence against deltamethrin-resistant bed bug strains [3], neither *kdr* target-site insensitivity nor cuticular thickening protected *C. lectularius* adults or nymphs from fungal colonization following a brief 15-minute contact window. Moreover, pre-lethal feeding inhibition (> 80% reduction within 24–48 h) neutralizes host biting well before Day-3 to Day-5 mortality peaks.

4.2 Gregarious "Trojan Horse" Amplification Against Cryptic Bed Bug Harborages

A recognized limitation of purely repellent surface treatments in bed bug management is the risk of dispersing foraging insects without eliminating cryptic harborages inside bed frames, baseboards, and wall voids. Integrating a mild gas-phase hydrosol plume ($RI = +68.8$–71.0%) with non-repellent, persistent fungal conidia resolves this limitation. While dipterans and host-seeking mosquitoes are strongly deterred from colonizing or ovipositing within the litter tray ($RI > +80$%), bed bugs or fleas that contact the litter perimeter—or encounter trace spore-bearing granules transiently adhered to feline pelage—acquire an infective conidial load. Because *C. lectularius* releases aggregation pheromones that enforce dense thigmotactic clustering within crevices, returning donors act as biological "Trojan horses," disseminating conidia across unexposed adults and nymphs [2] and achieving 84.0–90.0% colony mortality at a 1:5 donor-to-recipient ratio.

4.3 Selective Arthropod TRPA1 Irritation vs. Feline Hepatic and Mammalian Thermal Safety

From a veterinary toxicology perspective, the strict exclusion of concentrated essential oils is pivotal. Because *Felis catus* lacks functional hepatic UGT1A6 glucuronidation [4], lipophilic monoterpene oils pose unacceptable cumulative hepatotoxicity risks when cats groom litter residue from their digital pads. Steam-distilled aqueous *N. cataria* hydrosol (4.5%) delivers trace water-soluble nepetalactone sufficient to trigger insect TRPA1 irritant receptors [5] while remaining below the threshold for feline olfactory overstimulation or hepatic burden.

Equally critical is the thermodynamic biosafety barrier governing *B. bassiana*. Extensive toxicological evaluations confirm that *B. bassiana* is non-infectious to warm-blooded vertebrates [6]. Specifically, the patented *G. Bassiana* isolate exhibits optimal vegetative growth between $23\,^{\circ}$C and $28\,^{\circ}$C, with complete cessation of conidial germination and hyphal elongation at temperatures $\geq 34\,^{\circ}$C. Because normal feline and canine core body temperatures range between $38.0\,^{\circ}$C and $39.2\,^{\circ}$C (and human core temperature approximates $37.0\,^{\circ}$C), a physiological thermal margin of 4–$5\,^{\circ}$C strictly precludes fungal proliferation on or within mammalian hosts.

4.4 Methodological Implications and Future Field Surveillance

Because solid biopesticidal pet-care matrices fall outside standard liquid-spray insecticide testing guidelines, the four-stage bioassay architecture presented here provides a reproducible benchmark for evaluating multi-modal granular substrates. Ongoing deployment of the 30-day multi-cat household sticky-trap surveillance protocol across high-density Hong Kong apartments will further quantify longitudinal reductions in indoor arthropod pressure and long-term conidial shelf-life stability under fluctuating subtropical ambient humidity.

5 Conclusion

Integrating 4.5% essential-oil-free *Nepeta cataria* aqueous hydrosol and 0.5% dormant *Beauveria bassiana* conidia ($\geq 1 \times 10^8$ CFU/g) into a 95.0% pea-fiber tofu cat litter matrix establishes a highly effective, dual-layer biological pest defense system. The formulation achieves statistically significant gas-phase spatial repellency ($p < 0.001$) against nuisance dipterans, mosquitoes, fleas, and bed bugs, while delivering 94.0–100% direct-contact mortality and 84.0–90.0%

horizontal colony eradication against pyrethroid-resistant *Cimex lectularius*. Coupled with feline hepatic compatibility and a strict mammalian thermal barrier ($\geq 34\,^{\circ}$C growth arrest), this solid-matrix "Repel, Infect & Spread" platform offers a practical, chemical-free paradigm for integrated pest management in companion animal households.

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