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Efek Non-Target *Beauveria bassiana* dan *Metarhizium* spp. terhadap Artropoda Predator: Meta-Analisis Efek Letal dan Subletal pada Berbagai Jalur Paparan

Non-Target Effects of *Beauveria bassiana* and *Metarhizium* spp. on Predatory Arthropods: A Meta-Analysis of Lethal and Sublethal Effects Across Exposure Pathways

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ABSTRACT

Background: Entomopathogenic fungi (EPFs) such as *Beauveria bassiana* and *Metarhizium* spp. are widely used as biological control agents in integrated pest management (IPM), but their ecological risks to non-target predatory arthropods remain insufficiently understood; specifically, no quantitative synthesis has yet compared these effects across multiple exposure pathways and predator taxa simultaneously. **Objective**: This meta-analysis aimed to quantify and compare the lethal and sublethal effects of these two EPF species on predatory arthropods and to determine how exposure pathway and predator taxonomic group modulate the magnitude of ecological risk. **Methods:** Data were extracted from 27 laboratory studies published between 2015 and 2025, yielding 147 observations across more than 25 predator species representing six taxonomic orders (five with sufficient replication for pooled comparison). Effect sizes, expressed as log odds ratios and Hedges' g, were pooled using random-effects models, and mixed-effects moderator analyses compared EPF species, exposure pathway, and predator taxonomic order. **Results:** Both *B. bassiana* and *Metarhizium* spp. significantly increased predator mortality (pooled OR = 13.33) and reduced survival (OR = 7.665), with no significant difference in lethality between species. Developmental time was significantly prolonged and fecundity was significantly reduced, while longevity showed no significant overall effect, albeit based on limited data (k = 6). Exposure pathway was the strongest significant moderator of fecundity risk: direct application caused significantly greater fecundity reduction than soil or prey-mediated exposure, and Coleoptera showed the greatest susceptibility to developmental disruption among taxonomic orders based on the available data, with Coleoptera also being by far the most represented order (n = 67 observations). **Conclusions:** Exposure pathway, rather than EPF species identity, was the primary determinant of laboratory-observed lethal and sublethal effects on predatory arthropods, indicating that application method warrants priority attention in ecological risk assessment and in the design of EPF-based biological control strategies that seek to conserve predatory arthropods as key natural enemies, although field validation of these laboratory-based, worst-case-scenario estimates is still needed.

Keywords: *biological control; ecological risk; entomopathogenic fungi; Hedges' g; integrated pest management; natural enemy*

ABSTRAK

Pendahuluan: Cendawan entomopatogen (EPF) seperti *Beauveria bassiana* dan *Metarhizium* spp. banyak digunakan sebagai agens pengendali hayati dalam pengendalian hama terpadu (PHT), namun risiko ekologisnya terhadap artropoda predator non-target masih belum dipahami secara memadai; secara spesifik, belum ada sintesis kuantitatif yang membandingkan efek kedua spesies tersebut pada berbagai jalur paparan dan taksa predator secara simultan. **Tujuan**: Meta-analisis ini bertujuan mengkuantifikasi dan membandingkan efek letal dan subletal kedua spesies EPF tersebut terhadap artropoda predator, serta menentukan bagaimana jalur paparan dan kelompok taksonomi predator memodulasi besaran risiko ekologis. **Metode**: Data diekstraksi dari 27 studi laboratorium yang diterbitkan antara tahun 2015 dan 2025, menghasilkan 147 observasi dari lebih dari 25 spesies predator yang mewakili enam ordo taksonomi (lima di antaranya dengan replikasi memadai untuk perbandingan gabungan). Ukuran efek, berupa log odds ratio dan Hedges' g, digabungkan menggunakan model efek acak, dan analisis moderator efek campuran membandingkan spesies EPF, jalur paparan, dan ordo taksonomi predator. **Hasil**: Baik *B. bassiana* maupun *Metarhizium* spp. secara signifikan meningkatkan mortalitas predator (OR gabungan = 13,33) dan menurunkan kelangsungan hidup (OR = 7,665), tanpa perbedaan signifikan dalam letalitas antara kedua spesies. Waktu perkembangan memanjang secara signifikan dan fekunditas menurun secara signifikan, sementara umur (longevity) secara keseluruhan tidak terpengaruh secara signifikan, meskipun berdasarkan data yang terbatas (k = 6). Jalur paparan merupakan moderator signifikan terkuat untuk risiko terhadap fekunditas: aplikasi langsung menyebabkan penurunan fekunditas yang secara signifikan lebih besar dibandingkan paparan tanah atau melalui mangsa, dan Coleoptera menunjukkan kerentanan tertinggi terhadap gangguan perkembangan di antara ordo taksonomi berdasarkan data yang tersedia, dengan Coleoptera juga menjadi ordo yang paling banyak terwakili (n = 67 observasi). **Kesimpulan**: Jalur paparan, bukan identitas spesies EPF, merupakan determinan utama efek letal dan subletal yang teramati secara laboratoris pada artropoda predator, yang mengindikasikan bahwa metode aplikasi perlu mendapat perhatian prioritas dalam penilaian risiko ekologis dan perancangan strategi pengendalian hayati berbasis EPF yang berupaya mengkonservasi artropoda predator sebagai musuh alami utama, meskipun validasi lapangan terhadap estimasi berbasis laboratorium (skenario terburuk) ini masih diperlukan.

Kata kunci: *Cendawan entomopatogen; Hedges' g; musuh alami; pengendalian hama terpadu; pengendalian hayati; risiko ekologis*

BACKGROUND

Beauveria bassiana and *Metarhizium* spp. are among the most extensively studied and commercially deployed entomopathogenic fungi (EPFs) in biological control programs worldwide (Lacey et al., 2015; de Faria and Wraight, 2007). These filamentous fungi infect insect hosts through cuticle penetration, proliferate within the haemocoel, and ultimately cause host death through tissue disruption, nutrient depletion, and toxin production (Hajek and St. Leger, 1994; Hendra et al., 2026). Both genera share a broadly similar infection strategy: cuticle penetration mediated by extracellular hydrolytic enzymes followed by toxin production during haemocoel colonisation (Hajek and St. Leger, 1994), which provides a plausible mechanistic basis for comparable virulence toward non-target arthropods; however, genus- and strain-level differences in enzyme kinetics, toxin profiles, and host-range breadth have also been reported (Inglis et al., 2001; Goettel et al., 2010), so a priori expectations about whether the two genera should differ in non-target risk are not clear-cut. This mechanistic ambiguity is part of the motivation for empirically testing, rather than assuming, whether *B. bassiana* and *Metarhizium* spp. differ in their effects on predatory arthropods. Their broad host range, environmental persistence, and compatibility with other pest management inputs have made them central components of integrated pest management (IPM) systems across diverse cropping agroecosystems globally (Lacey et al., 2015; van Lenteren et al., 2018).

Despite their ecological advantages over synthetic insecticides, EPFs are not inherently selective and may affect non-target organisms co-occurring in the same agroecosystem (Furlong and Pell, 2005; Roy and Cottrell, 2008). Predatory arthropods, including coccinellid beetles, phytoseiid and stigmaeid predatory mites (Acari), green lacewings (Neuroptera: Chrysopidae), predatory bugs (Hemiptera: Anthocoridae and Miridae), and spiders (Araneae), represent the most ecologically consequential non-target guild in agricultural fields (Eilenberg et al., 2001). These natural enemies provide indispensable ecosystem services by suppressing herbivorous pest populations, reducing dependence on pesticide applications, and sustaining the efficacy of augmentative and conservation biological control programs (van Lenteren et al., 2018). Their exposure to EPFs in the field may occur through several routes, including direct contact with aerosolized conidia during spray application, ingestion of EPF-infected prey, and contact with fungal residues persisting on plant surfaces or in soil (Meyling and Eilenberg, 2006; Bruck, 2010).

Laboratory bioassays have frequently documented both lethal and sublethal effects of EPFs on predatory arthropods. Lethal effects include elevated mortality and reduced survival rates following conidial exposure, while sublethal consequences encompass impaired fecundity, prolonged developmental time, reduced predation rate and feeding efficiency, and altered longevity (Roy and Cottrell, 2008; Ullah and Lim, 2017; Rizwan et al., 2021). However, the magnitude and direction of these effects vary considerably across individual studies, attributable to differences in EPF species and strain virulence, conidial concentration, predator species and life stage, and the mode of exposure applied in experimental assays (Inglis et al., 2001; Goettel et al., 2010). Of particular importance is the exposure pathway, specifically whether through direct topical application, prey-mediated ingestion, foliar contact, or soil exposure, which may fundamentally determine the ecological risk posed under realistic field conditions, where conidial degradation, predator avoidance behaviour, and dilution effects reduce actual conidial contact (Klingen and Haukeland, 2006; Roy et al., 2006).

Despite the accumulation of individual bioassay studies, and to our knowledge based on the systematic multi-database search described in Methods, no quantitative synthesis has yet comprehensively compared the effects of *B. bassiana* and *Metarhizium* spp. across multiple exposure pathways and response variables simultaneously. Prior narrative reviews have usefully catalogued EPF effects on natural enemies qualitatively (Roy and Cottrell, 2008; Furlong and Pell, 2005; Meyling and Eilenberg, 2006), but they do not yield standardized effect size estimates necessary for rigorous cross-study comparison, moderator analysis, or quantitative risk ranking. Without such synthesis, critical questions remain unresolved: whether the two most widely deployed EPF genera pose comparable ecological risks to predatory arthropods; which exposure pathways drive the greatest harm to predator reproduction and survival; and whether specific predator taxonomic groups are disproportionately susceptible; all of which constitute information essential for evidence-based ecological risk assessment in IPM. Because the available evidence base is composed of laboratory bioassays, this meta-analysis quantifies laboratory-derived lethal and sublethal effects; these findings inform, but do not by themselves establish, ecological risk under field conditions, where exposure intensity, conidial persistence, predator avoidance behaviour, and habitat complexity may substantially modify outcomes. Throughout this manuscript, the term "ecological risk" is therefore used in a specific, narrow sense: it denotes the laboratory-demonstrated potential for EPF exposure to harm non-target predatory arthropods (a laboratory-based non-target toxicity risk), which serves as a starting point and upper-bound indicator for, but is not equivalent to, field-validated ecological risk.

Meta-analysis provides a statistical framework that addresses these gaps directly, enabling pooling of effect sizes across heterogeneous studies, identification of key moderating variables through mixed-effects modelling, and formal assessment of publication bias (Borenstein et al., 2009; Koricheva et al., 2013). Applied to ecological risk assessment, meta-analytic quantification translates accumulated qualitative evidence into actionable, magnitude-specific guidance for EPF deployment strategies.

Therefore, this meta-analysis aimed to: (1) quantify and compare the lethal and sublethal effects of *B. bassiana* and *Metarhizium* spp. on predatory arthropods across five response variables (mortality, survival, developmental time, fecundity, and longevity); (2) determine whether exposure pathway (direct application, prey-mediated, foliar, or soil) significantly moderates the magnitude of these effects; (3) assess variation in susceptibility across predator taxonomic orders; and (4) synthesize findings, together with their laboratory basis and limitations, into pathway-specific risk indications that can inform (rather than directly prescribe) EPF-based IPM programs seeking to conserve predatory arthropods as key natural enemy guilds.

METHODS

Study Selection and Database Search

This meta-analysis followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (Page et al., 2021). Articles were retrieved from Scopus and PubMed using the following search terms: (“*Beauveria bassiana*” OR “*Metarhizium* spp.” OR “*Metarhizium robertsii*” OR “*Metarhizium brunneum*” OR “entomopathogenic fung*”) AND (“predator*” OR “natural enem*” OR “predatory arthropod*” OR “biological control agent*” OR “Coccinellidae” OR “Chrysopidae” OR “Acari”) AND (“mortalit*” OR “surviv*” OR “fecundit*” OR “longevit*” OR “developmental time” OR “sublethal” OR “non-target” OR “ecological risk”). The search was limited to English-language peer-reviewed articles published between 2015 and 2025. Studies were included if they: (i) tested *B. bassiana* or *Metarhizium* spp. on predatory arthropods; (ii) reported at least one quantitative measure of lethal or sublethal effect; (iii) included a control group; and (iv) provided sufficient data for effect size calculation (mean ± SD or SE, and sample size, or proportion with sample size). Conversely, studies were excluded if they did not meet all four criteria, for example, if they tested EPF species other than *B. bassiana* or *Metarhizium* spp., did not include predatory arthropods, lacked an untreated/control comparison group, or reported effects only qualitatively without data sufficient for effect size calculation.

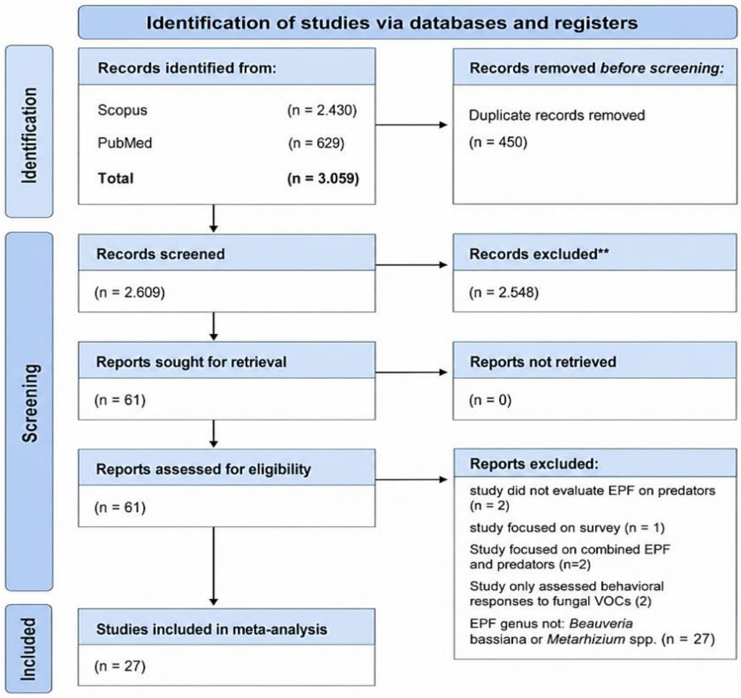

Figure 1. PRISMA 2020 flow diagram illustrating the study selection and screening process. Records were identified from two electronic databases (Scopus and PubMed). Final inclusion criteria yielded 27 studies comprising 147 observations.

The systematic search and screening process yielded a total of 27 eligible studies comprising 147 observations that met all inclusion criteria. Studies were published between 2015 and 2025 and conducted predominantly under laboratory conditions across multiple countries. Predatory arthropods represented six taxonomic orders: Coleoptera, Acari, Hemiptera, Neuroptera, Araneae, and Diptera; Diptera was represented by only a single observation and was therefore excluded from formal pooled and moderator comparisons, which were based on the remaining five orders. Both EPF species were well represented across all response variables. The distribution of observations across predator orders and response variables is presented in Supplementary Figure S7, revealing that Coleoptera was the most studied order (n = 67 obs) and mortality the most frequently tested endpoint

Data Extraction

Data were extracted by the first author and verified against the original source articles. For each study, the following information was recorded: fungal species, conidial concentration, predator species and taxonomic order, life stage tested, exposure pathway (direct application, prey-mediated, foliar application, environmental contamination, soil application, oral ingestion), response variable (mortality, survival, feeding behaviour/predation rate, fecundity, longevity, developmental time, reproduction), study setting (laboratory, semi-field, field), and country of origin. Data presented only in figures were extracted using WebPlotDigitizer v.4.6 (Rohatgi, 2022).

Response variables were categorized into four main groups: (1) mortality, (2) survival, (3) feeding behaviour/predation, and (4) sublethal reproductive and developmental effects (fecundity, longevity, developmental time). Mortality and survival were treated as distinct, non-overlapping outcome categories rather than as a single event coded two ways: mortality observations (k = 29) were drawn from studies reporting the number or proportion of individuals that died following EPF exposure over a defined observation period, whereas survival observations (k = 31) were drawn from studies reporting the number or proportion of individuals remaining alive, typically assessed at one or more specific time points or via survival curves; where a study reported both, each was extracted as a separate observation in its respective category rather than derived from one another. Feeding behaviour/predation data were extracted but were reported across studies in highly heterogeneous units and formats (e.g., handling time, prey consumed, proportion feeding), yielding only k = 4 usable observations after standardisation; these data were therefore not subjected to formal meta-analytic pooling and are instead summarised narratively in the Discussion. In this meta-analysis, an "observation" is defined as one extracted effect size, corresponding to a single species × fungal isolate/strain × exposure pathway × response variable combination reported in a given study; a single study could therefore contribute multiple observations when it tested more than one such combination.

To limit non-independence among observations drawn from the same study, only one treatment was retained per species × fungus × exposure pathway combination within a study, following the selection rule described immediately below, so that repeated measurements or closely related treatment arms from the same experiment were not entered as separate, fully independent observations.

We acknowledge that this study-level de-duplication reduces but does not fully eliminate residual dependence among observations that share a source study, predator species, or fungal isolate; formal accounting for this structure (e.g., via robust variance estimation or multilevel models with random effects for study) was not implemented in the present analysis and is noted as a limitation. Where multiple treatments (e.g., concentrations or doses) were reported per study for the same species × fungus × exposure pathway combination, only the data from the highest tested concentration or dose were used, following a predefined worst-case selection rule applied consistently across studies; this treatment also generally corresponds to the one for which effects were most consistently and completely reported.

Effect Size Calculation and Statistical Analysis

Effect sizes were calculated using Hedges' g for continuous data (means-based) and log odds ratios for binomial/proportion data, which were analysed using the appropriate effect size metric for each data type (log OR for proportion data; Hedges' g for continuous data). All analyses were performed using R version 4.4.2 using the 'metafor' package (Viechtbauer, 2010). Mixed-effects models with categorical moderators were fitted to compare effect sizes between: (1) EPF species (*B. bassiana* vs *Metarhizium* spp.), (2) exposure pathway (direct, prey-mediated, foliar, other), (3) taxonomic order of predator, and (4) response variable type. EPF species was tested as a moderator for all five response variables.

Exposure pathway was tested for mortality, developmental time, and fecundity; foliar and residual-contamination pathways were represented only among survival and feeding-behaviour observations, which were not subjected to pathway moderator analysis, and within each tested response variable, pathway categories represented by fewer than two independent observations were included in the omnibus moderator test but not displayed as separate pooled subgroups in the corresponding figure. Taxonomic order was tested for mortality, developmental time, and fecundity; it could not be reliably tested for longevity, for which the available observations (k = 6) were too sparsely distributed across taxonomic orders (one to two observations per group) to support a statistically meaningful comparison.

Effect sizes were considered significantly different from zero when 95% confidence intervals (CI) did not overlap with zero. Groups were considered significantly different from each other when their 95% CIs did not overlap; however, this non-overlap criterion was treated only as a secondary, descriptive indicator, since it is conservative and does not itself constitute a formal statistical test. The primary basis for concluding that groups differed was the omnibus moderator (QM) test reported for each moderator; where the QM test was not significant, apparent contrasts between individual subgroups are reported as descriptive only and are explicitly flagged as not statistically confirmed.

Heterogeneity among studies was assessed using the Q statistic and I^2 index (Higgins and Thompson, 2002). For mixed-effects moderator models, R^2 was computed as the proportional reduction in the estimated between-study heterogeneity variance (τ^2) attributable to the moderator, relative to the corresponding random-effects model without moderators (i.e., the percentage of otherwise-unexplained between-study variance accounted for by that moderator); it is therefore reported only in conjunction with a significant QM test and should not be interpreted as a variance-explained statistic in the ordinary-regression sense. Publication bias was evaluated using funnel plot asymmetry and Egger's test (Egger et al., 1997). Funnel plot asymmetry was not significant for mortality (z = 1.60, p = 0.122) or fecundity (z = 0.68, p = 0.517), indicating no significant publication bias for these variables. However, significant asymmetry was detected for survival (z = 4.79, p < 0.001) and developmental time (z = 4.06, p = 0.001), likely attributable to small-study effects and heterogeneity in experimental designs rather than selective reporting. Longevity (k = 6) had insufficient power for formal Egger's test.

RESULTS AND DISCUSSION

Overall Effects of EPF on Predatory Arthropods

The effects of EPF across all five response variables are summarised in Figure 2. Both *B. bassiana* and *Metarhizium* spp. significantly increased predatory arthropod mortality (Hedges' g = 1.428, 95% CI: 1.109–1.748, p < 0.001, n = 29; equivalent to a pooled odds ratio of OR = 13.33, 95% CI: 7.457–23.829, converted following Borenstein et al., 2009) and reduced survival (g = 1.123, 95% CI: 0.807–1.439, p < 0.001, n = 31; equivalent to OR = 7.665, 95% CI: 4.322–13.593). Developmental time was significantly prolonged (g = 2.023, 95% CI: 1.262–2.784, n = 16), while fecundity was significantly reduced (g = −0.861, n = 10). In contrast, longevity showed no significant overall effect (g = −1.189, p = 0.119, n = 6), although this result was based on a limited number of observations.

The approximately 13-fold increase in the odds of mortality indicates a substantial overall adverse effect of EPF exposure under the conditions represented in the dataset. This is consistent with previous qualitative reviews reporting lethal and sublethal effects of EPFs on non-target predators (Roy and Cottrell, 2008; Furlong and Pell, 2005), but the present meta-analysis extends these observations by providing standardized effect-size estimates that allow quantitative comparison. A comparable meta-analytic approach applied to herbicide non-target effects on natural enemies likewise found consistently elevated mortality and reduced longevity, reproduction, and predation efficacy relative to controls, suggesting that quantitative synthesis can reveal disruption patterns that narrative reviews may not adequately capture, whether the agent is a biopesticide or a conventional agrochemical (Zilnik et al., 2023).

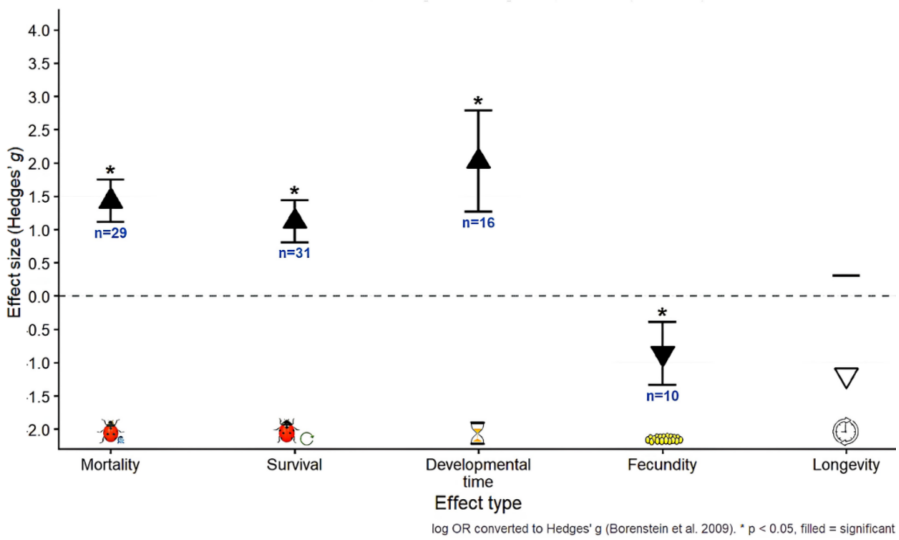

Figure 2. Effect sizes (Hedges' g) for each response variable tested in EPF non-target effects assays on predatory arthropods. Error bars indicate 95% confidence intervals. Filled symbols indicate significant effects (p < 0.05); open symbols indicate non-significant effects. Upward triangles = positive effects (more mortality/longer development); downward triangles = negative effects (reduced fecundity/longevity). n = number of observations. log OR converted to Hedges' g (Borenstein et al., 2009)

The pooled mortality effect was particularly pronounced (OR = 13.33, 95% CI: 7.457–23.829), indicating that EPF-treated predatory arthropods had approximately 13-fold higher odds of death than controls across the combined exposure pathways. When restricted to direct application, the effect was somewhat smaller (g = 1.635, n = 23), indicating that direct exposure represents one important contributor to the overall pooled mortality effect rather than its sole basis. Despite the large overall effect, no significant difference in lethality was detected between *B. bassiana* (g = 1.491) and *Metarhizium* spp. (g = 1.356; $Q_m(1) = 0.232$, p = 0.630), suggesting broadly comparable effects of both EPF genera on predatory arthropods.

A direct within-study comparison from Mohamed (2019) supports this pattern: both fungi caused relatively high adult mortality in the same coccinellid, *Cryptolaemus montrouzieri*, with mortality of 38.6% for *B. bassiana* and 29.5% for *M. anisopliae*. The comparable effect sizes observed in the present meta-analysis may reflect similarities in infection mechanisms and host-range breadth between these EPF genera (Inglis et al., 2001; Goettel et al., 2010).

Survival was likewise significantly reduced following EPF exposure (OR = 7.665, 95% CI: 4.322–13.593). Both EPF genera produced significant survival reductions, with no significant difference between *B. bassiana* and *Metarhizium* spp. ($Q_m(1) = 0.782$, p = 0.377). Individual studies in the dataset further illustrate this general pattern. Dogan et al. (2017) recorded up to 99.5% mortality of the predatory mite *Phytoseiulus persimilis* following exposure to *Metarhizium brunneum*, whereas Wu et al. (2015) documented significantly reduced longevity and fecundity in Neoseiulus barkeri following prey-mediated exposure to *B. bassiana*. Sayed et al. (2021) also reported significant developmental delays in *Coccinella undecimpunctata* and *Hippodamia variegata* after direct application of *B. bassiana*.

Although feeding behaviour/predation was not subjected to formal meta-analytic pooling because only four usable observations were available and the data were reported in highly heterogeneous units and formats, individual studies provide evidence that EPF exposure can influence predator behaviour. Ríos-Moreno et al. (2018) found that *Metarhizium brunneum* posed an overall low ecotoxicological risk to *Chrysoperla carnea*, although sublethal behavioural responses were still detectable. This is broadly consistent with reports of sublethal interference with prey detection, handling capacity, and predation rate in EPF-exposed predators (Ullah and Lim, 2017; Rizwan et al., 2021). Such behavioural changes may have ecological consequences for biological control services even when predators survive the initial EPF exposure. The limited availability of standardized feeding-behaviour data therefore represents an important gap in the current literature.

Life Stage Susceptibility

The comparison of EPF effects between adult and juvenile (larva/nymph) life stages for mortality and survival is presented in Figure 3. Both adults and juveniles showed significantly elevated mortality and reduced survival when exposed to EPF. For mortality, adults (g = 1.78, n = 16) showed a higher effect size than juveniles (g = 1.00, n = 13), although both were significant. For survival, both adults (g = 0.80, n = 20) and juveniles (g = 1.30, n = 3) showed significant survival reductions, with juveniles showing a numerically larger effect. However, the very small number of juvenile observations for survival warrants cautious interpretation. The overall susceptibility of both life stages indicates that EPF effects are not restricted to a particular developmental stage.

The higher numerical mortality effect in adults may reflect differences in exposure or physiological characteristics among the species represented in the dataset, whereas the apparently greater survival reduction in juveniles should not be interpreted as evidence of greater juvenile susceptibility because it was based on only three observations. Thus, life-stage comparisons suggest broad susceptibility across developmental stages, but additional standardized studies are needed to determine whether susceptibility consistently varies with predator age.

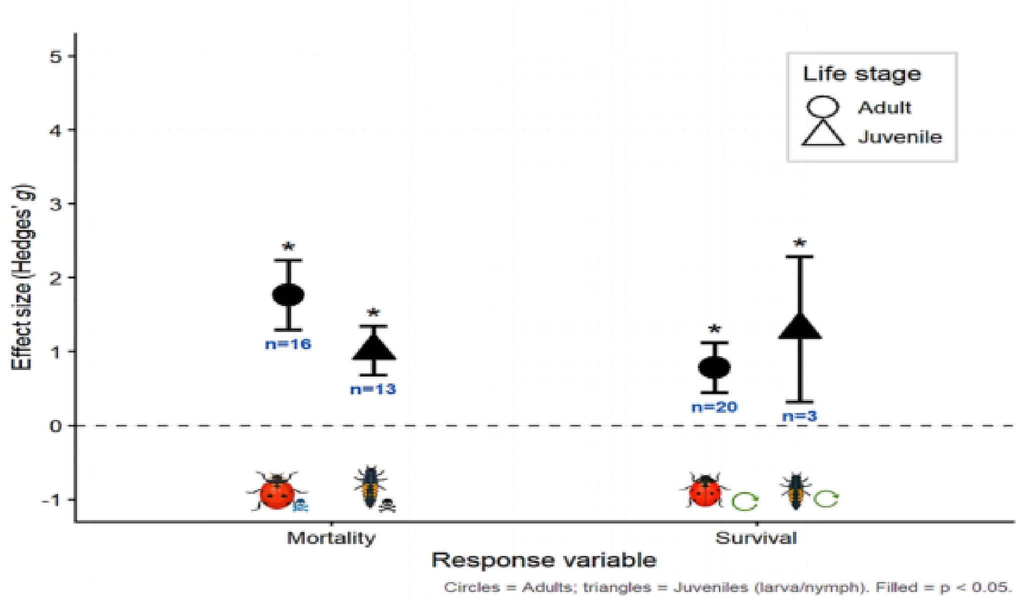

Figure 3. Effect sizes (Hedges' g) of EPF-caused mortality and survival reduction in adult (circles) and juvenile (triangles) predatory arthropods. Mortality: adults n=16, juveniles n=13. Survival: adults n=20, juveniles n=3. Error bars = 95% CI. Filled = p < 0.05.

EPF Species Comparison and Exposure Pathway Effects

Figure 4 compares the effect sizes of *B. bassiana* and *Metarhizium* spp. across all five response variables. No significant difference in overall lethality was detected between the two EPF groups ($Q_m(1) = 0.232$, $p = 0.630$ for mortality), indicating broadly comparable ecological risk from both genera. Both *B. bassiana* and *Metarhizium* spp. consistently produced significant positive effects on mortality and developmental time and significant negative effects on fecundity. For survival, *B. bassiana* (n = 23, g = 1.206 [0.883; 1.529]) and Metarhizium spp. (n = 8, g = 0.979 [0.212; 1.747]) both showed significant survival reduction, with no significant difference between genera ($Q_m(1) = 0.782$, $p = 0.377$). Longevity was non-significant for both *B. bassiana* (g = −1.219, n = 4) and *Metarhizium* spp. (g = −1.054, n = 2).

The lack of significant differences between the two EPF genera suggests that the non-target risk to predatory arthropods is not confined to a single fungal genus. Rather, both genera can produce comparable lethal and sublethal effects depending on the response variable and exposure conditions. This interpretation is consistent with the similar mortality effects observed in the pooled analysis and with the within-study comparison reported by Mohamed (2019). However, differences among fungal strains, concentrations, exposure durations, and predator species may still influence the magnitude of effects within each genus.

Exposure pathway was a significant moderator for fecundity ($Q_m(3) = 16.82$, $p < 0.001$, $R^2 = 71.0\%$), with direct application producing the greatest reduction in fecundity (g = −1.314). Direct application was the only pathway associated with significant fecundity reduction (g = −1.314, p < 0.001, n = 5), whereas soil exposure showed no significant effect (n = 3). For mortality, direct application produced a significant effect (g = 1.635, n = 23), whereas soil exposure was not significant (n = 4). Prey-mediated exposure produced a significant effect on developmental time (g = 1.779, p < 0.001, n = 9).

The significant moderator effect for fecundity indicates that exposure pathway is particularly important for reproductive responses. The strong reduction associated with direct application is biologically plausible because direct spraying or immersion maximizes contact between fungal conidia and the arthropod cuticle, whereas soil exposure may involve environmental buffering and dilution. However, exposure pathway should not be interpreted as an isolated causal factor because conidial concentration, exposure duration, fungal strain, predator species, and life stage were not evenly distributed among pathway categories. Consequently, part of the observed pathway effect may reflect correlated study-level differences.

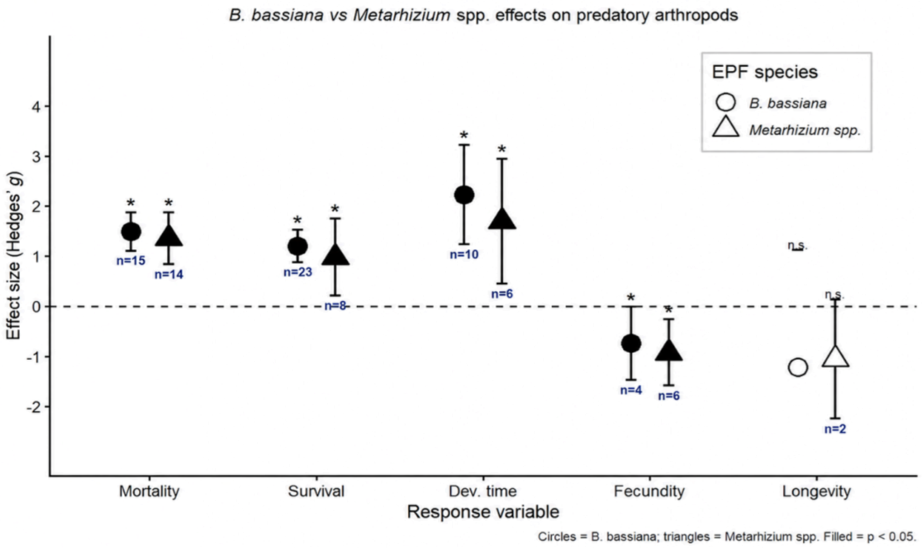

Figure 4. Effect sizes (Hedges' g) of *B. bassiana* and *Metarhizium* spp. across five response variables (mortality, survival, developmental time, fecundity, and longevity). Circles = *B. bassiana*; triangles = *Metarhizium* spp. Error bars = 95% CI. Filled symbols = $p < 0.05$; n.s. = not significant. n = number of observations. No significant difference between the two EPF genera was detected for mortality ($Q_m(1) = 0.232$, $p = 0.630$) or survival ($Q_m(1) = 0.782$, $p = 0.377$).

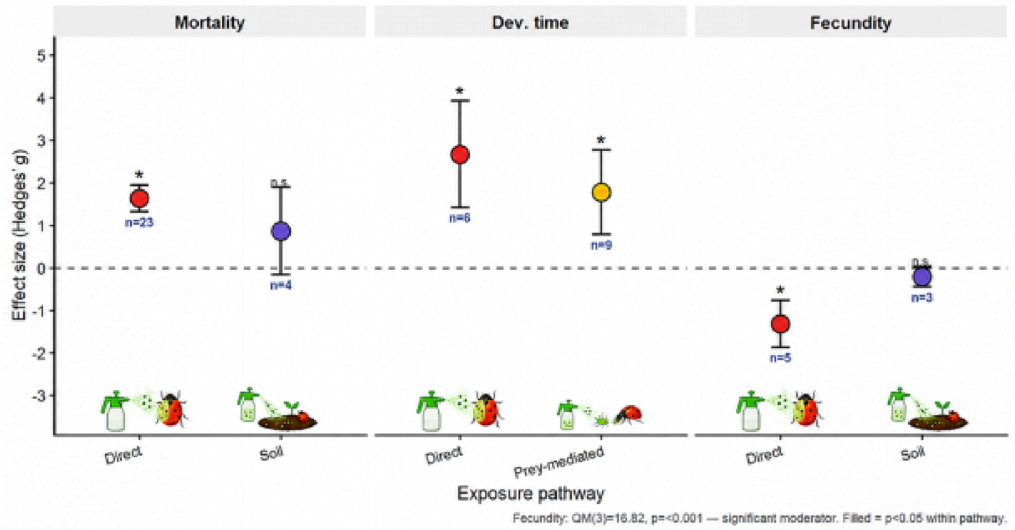

Figure 5. Effect of exposure pathway on EPF risk to predatory arthropods across three response variables (mortality, developmental time, fecundity). Each panel shows pooled effect sizes per pathway; error bars = 95% CI. Filled circles = $p < 0.05$ within pathway. Direct application produced the largest fecundity reduction ($g = -1.314$). Fecundity was the only variable for which exposure pathway was a significant moderator ($QM(3) = 16.82$, $p < 0.001$, $R^2 = 71.0\%$)

The direct-application effect also represents an important consideration for ecological risk assessment. Direct application can represent a worst-case exposure scenario and may overestimate risks under field conditions, where conidial degradation, avoidance behaviour, and dilution can reduce actual contact (Bruck, 2010; Klingen and Haukeland, 2006). Consistent with this interpretation, de Azevedo et al. (2019) found that soil-applied *M. anisopliae* produced comparatively modest and taxon-dependent effects on *Orius majusculus, Dalotia coriaria,* and *Aphidoletes aphidimyza,* relative to the near-total mortality commonly reported under direct exposure. Similarly, prey-mediated studies by Ullah et al. (2019) and Sahayaraj et al. (2018) reported sublethal effects on handling time and nymphal development in reduviid predators rather than acute mortality.

Prey-mediated exposure remains ecologically relevant because predatory arthropods may consume EPF-infected prey under field conditions (Roy et al., 2006). Behavioural avoidance may further reduce realized exposure. Bayissa et al. (2016), for example, observed that the coccinellid Cheilomenes lunata actively avoided aphids infected with M. anisopliae, which could reduce actual conidial contact under field conditions compared with forced-exposure laboratory assays. Thus, the risk associated with EPF exposure is likely to depend not only on fungal identity but also on how and under what environmental conditions predators encounter the fungus.

For mortality, however, the corresponding omnibus moderator test did not reach significance ($Q_m(3) = 6.27$, $p = 0.099$). Therefore, although direct application produced a larger numerical effect than soil exposure, this contrast should be interpreted as suggestive rather than statistically confirmed.

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Taxonomic Variation in Susceptibility

The effects of EPF on mortality differed numerically across predator taxonomic orders, as shown in Figure 6. The dataset included Acari (n = 3), Araneae (n = 2), Coleoptera (n = 18), Diptera (n = 1), Hemiptera (n = 2), and Neuroptera (n = 3). All orders with more than one observation showed positive effect sizes, indicating increased mortality across groups: Hemiptera showed the largest numerical pooled effect (g = 2.08, n = 2), followed by Araneae (g = 1.84, n = 2), Acari (g = 1.74, n = 3), Neuroptera (g = 1.55, n = 3), and Coleoptera (g = 1.30, n = 18). Diptera was represented by a single observation (g = 0.42) and was therefore interpreted cautiously because pooling was not possible.

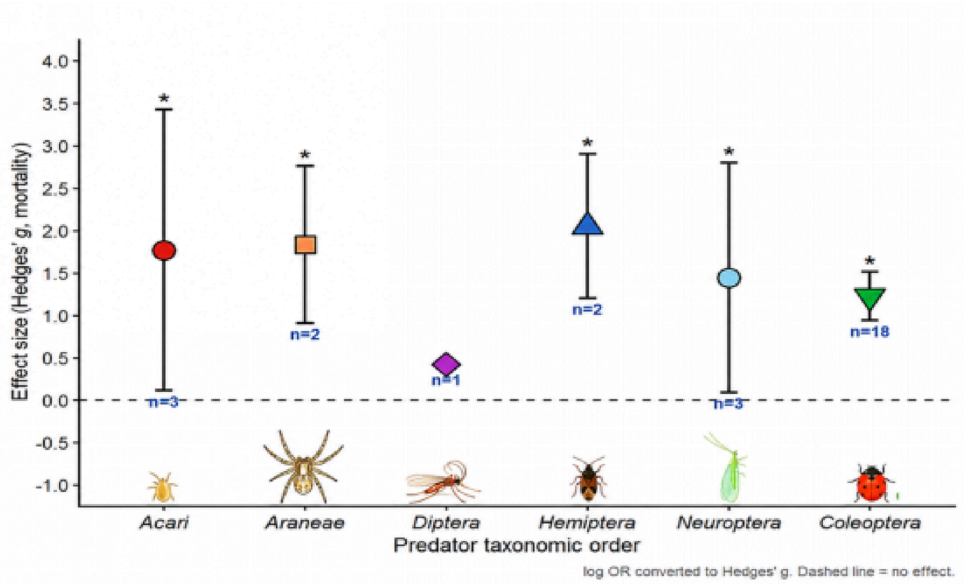

Figure 6. Effect sizes (Hedges' g) of EPF-caused mortality across predator taxonomic orders. Each symbol represents the pooled estimate for that order; error bars = 95% CI. Filled symbols = $p < 0.05$. n = number of observations. Diptera (n = 1) shown without pooling. log OR converted to Hedges' g.

Despite these numerical differences, the overall moderator test did not detect a significant difference among taxonomic orders ($Q_m(5) = 4.30$, $p = 0.508$). Therefore, the numerical ranking should not be interpreted as evidence that Hemiptera, Araneae, Acari, or Neuroptera are more susceptible than Coleoptera. The small number of observations in several groups means that individual studies may substantially influence pooled estimates. The absence of a significant taxonomic moderator suggests that lethal effects of EPFs are broadly distributed across predator groups, although statistical power was limited for several orders.

In contrast, taxonomic order significantly moderated developmental time effects ($Q_m(3) = 12.13$, $p = 0.007$), with Coleoptera exhibiting the largest developmental prolongation (g = 3.06, n = 8), followed by Acari (g = 1.66, n = 1), Hemiptera (g = 0.96, n = 6), and Neuroptera (g = 0.38, n = 1). This pattern suggests that sublethal developmental disruption may be more taxon-specific than lethal effects. Differences among taxonomic groups may reflect variation in cuticle composition, grooming behaviour, immune responses, and detoxification capacity (Hajek and St. Leger, 1994).

The present results are supported by individual studies. Trizelia et al. (2017) found prolonged larval-to-adult development in the coccinellid Menochilus sexmaculatus following exposure to *Metarhizium* spp., while Atrchian et al. (2022) reported comparable developmental delays in the same species following M. anisopliae exposure, despite non-significant effects on fecundity in both studies. Predatory mites (Mesostigmata) have also been reported to rank among the more sensitive natural-enemy taxa to insecticides, fungicides, and herbicides (Schmidt-Jeffris et al., 2021). This broader sensitivity across pest-management products suggests that some arthropod lineages may be inherently vulnerable to multiple classes of control agents rather than being uniquely sensitive to EPFs. The developmental vulnerability observed in Coleoptera is particularly relevant for EPF deployment because coccinellids and other beetles are widely used as biological control agents.

Taxonomic order was also tested as a moderator for fecundity but did not reach significance ($Q_m(3) = 2.29$, $p = 0.514$). The pooled effects were $g = -1.21$ for Acari ($n = 4$), $g = -0.81$ for Coleoptera ($n = 4$), $g = -0.27$ for Hemiptera ($n = 1$), and $g = -0.10$ for Neuroptera ($n = 1$), indicating broadly consistent fecundity reductions across the represented taxonomic groups. Taxonomic order could not be reliably tested as a moderator for longevity because the available observations ($k = 6$) were distributed across four orders with only one to two observations per group, leaving insufficient residual degrees of freedom for a statistically meaningful comparison.

Implications and Limitations

Collectively, the findings indicate that EPF exposure can impose substantial ecological risks to predatory arthropods, particularly through increased mortality, reduced survival, prolonged development, and reduced fecundity. Among the moderators examined, exposure pathway showed the clearest response-specific influence, particularly for fecundity, whereas EPF species and predator taxonomic identity produced more limited or outcome-dependent differences. From an integrated pest management (IPM) perspective, these findings support the consideration of application strategies that minimize direct contact between EPFs and predatory arthropods, such as soil application or endophytic colonization. Previous literature suggests that such approaches can maintain pest-control efficacy (Lacey et al., 2015; van Lenteren et al., 2018), although the present meta-analysis did not directly evaluate target-pest efficacy.

Nevertheless, the results should be interpreted in light of several limitations. Sample sizes were unevenly distributed across taxonomic orders and response variables. Coleoptera dominated the dataset overall ($n = 67$ observations), whereas Araneae and Hemiptera contributed only one to two observations each, and longevity was represented by only $k = 6$ observations. This imbalance limits statistical power for subgroup and moderator comparisons. Consequently, non-significant findings, including the taxonomic-order moderator for mortality ($Q_m(5) = 4.30$, $p = 0.508$) and the overall longevity effect, should not be interpreted as definitive evidence of biological equivalence or absence of effects.

Heterogeneity among studies also remained high, with I^2 values generally exceeding 80% even after accounting for the moderators tested. Funnel plot asymmetry was statistically significant for survival and developmental time, indicating possible small-study effects and additional unmeasured study-level variation. These factors warrant caution when generalizing pooled estimates to field conditions. In addition, the search was restricted to English-language, peer-reviewed articles published between 2015 and 2025. Residual non-independence among observations sharing a source study, predator species, or fungal isolate was only partially addressed through study-level de-duplication rather than formal multilevel or robust-variance modelling.

A sensitivity analysis excluding ATR2022 at LC20 concentration nevertheless confirmed the robustness of the main conclusions: all significant effects remained significant and effect-size magnitudes changed minimally (Table S1). Detailed per-study forest plots for each response variable are provided in Supplementary Figures S1–S5. Overall, the consistency of the main findings across the sensitivity analysis, together with the significant lethal and sublethal effects observed across multiple predator groups, indicates that EPF deployment should incorporate non-target risk assessment, particularly for reproductive and developmental effects and under exposure scenarios involving direct contact.

CONCLUSION

This meta-analysis demonstrates that *B. bassiana* and *Metarhizium* spp. produce comparable laboratory-derived lethal and sublethal effects on predatory arthropods, significantly increasing mortality (pooled OR = 13.33) and reducing survival, developmental time, and fecundity, with no significant overall effect on longevity (albeit based on limited data, k = 6). Exposure pathway, rather than fungal species or predator taxonomic identity, was the strongest significant driver of these effects, particularly for fecundity: direct application caused substantially greater reproductive harm than soil or prey-mediated exposure, and Coleoptera showed disproportionate susceptibility to developmental disruption. These results directly address the study's aims by quantifying and comparing lethal and sublethal EPF effects across exposure pathways and predator taxa, providing standardized effect sizes that can inform, but do not by themselves constitute, ecological risk assessment. Because the evidence base is laboratory-derived, these findings should be interpreted as an upper-bound indication of risk under realistic field conditions, where conidial degradation, predator avoidance behaviour, and habitat complexity are expected to reduce actual exposure and effect magnitude. For EPF-based IPM programmes, minimising direct contact through soil application, seed treatment, or endophytic colonisation may reduce non-target harm to predatory arthropods; whether these alternative application methods maintain equivalent efficacy against the target pest was not evaluated in this meta-analysis and remains an important question for future research. Future research should prioritise ecologically realistic exposure scenarios and field validation across a broader range of predator taxa and EPF formulations.

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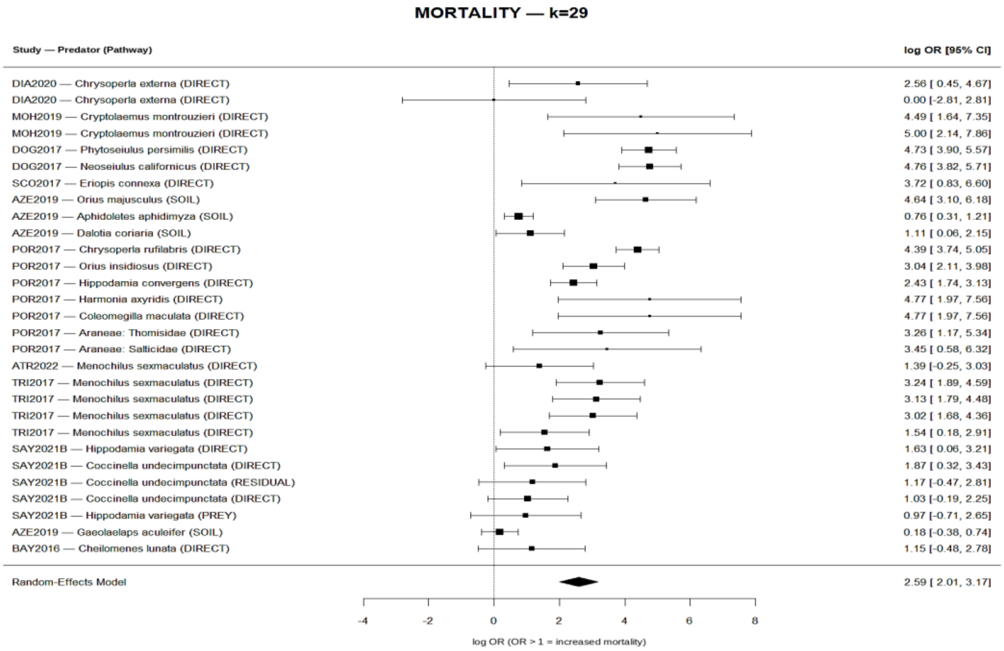
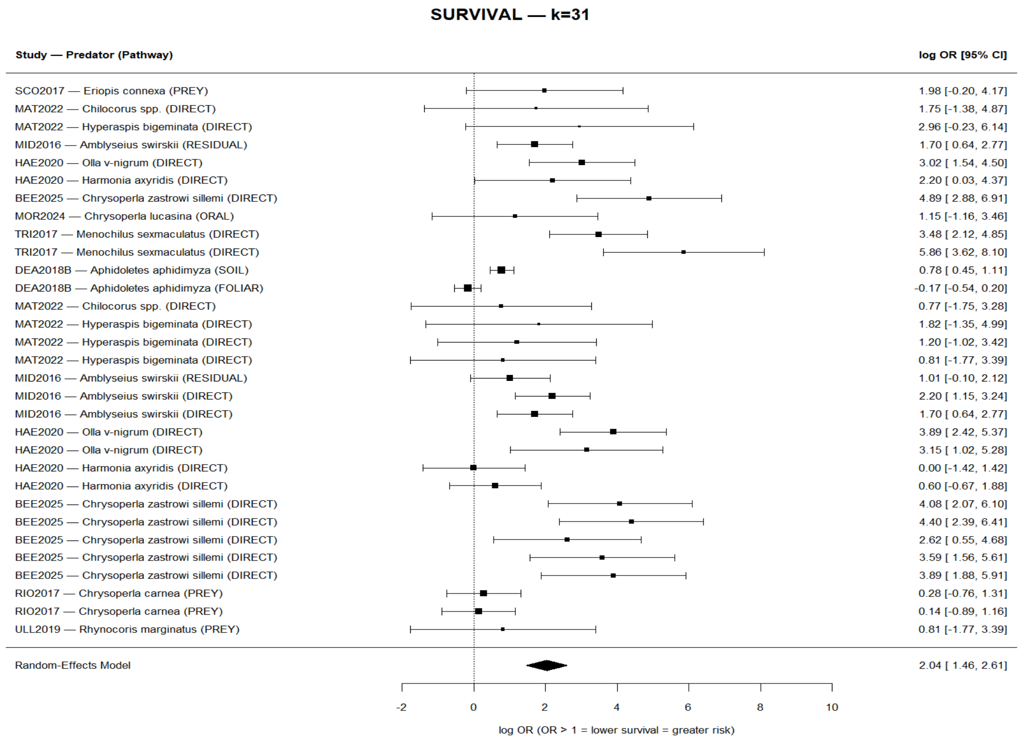
SUPPLEMENTARY MATERIAL

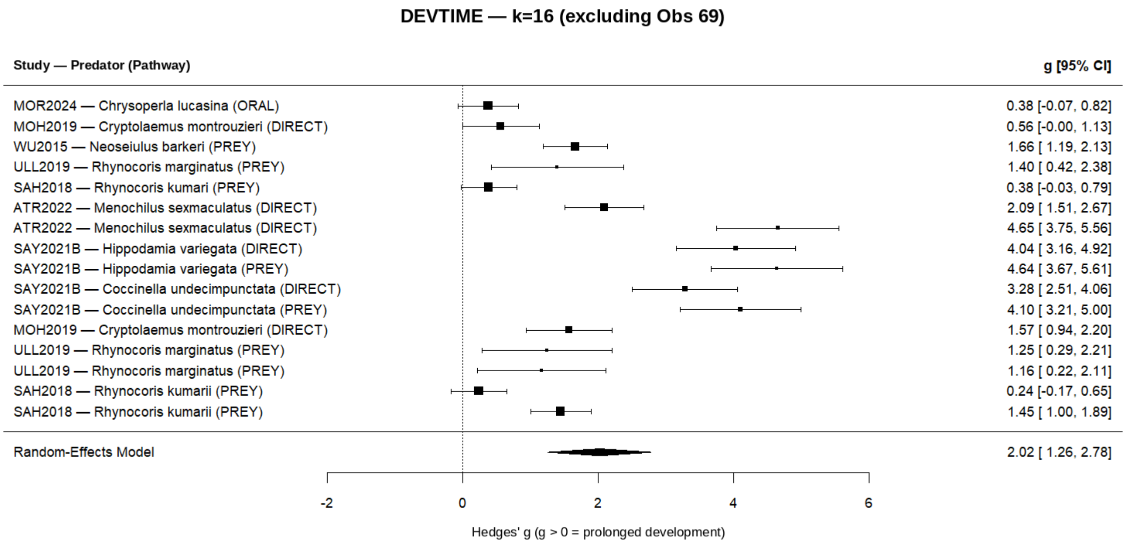
The following supplementary figures provide detailed forest plots and additional analyses supporting the main results.



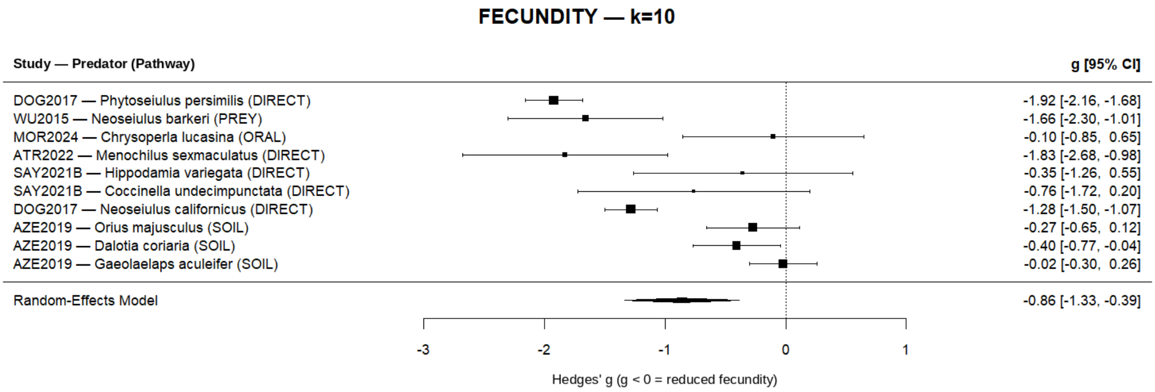
Supplementary Figure S1. Forest plot of mortality effects (log Odds Ratio) of EPF on predatory arthropods (k = 29 observations). OR > 1 = higher odds of mortality in EPF-treated group. Diamond = pooled random-effects estimate (OR = 13.33, 95% CI: 7.457–23.829, p < 0.001).



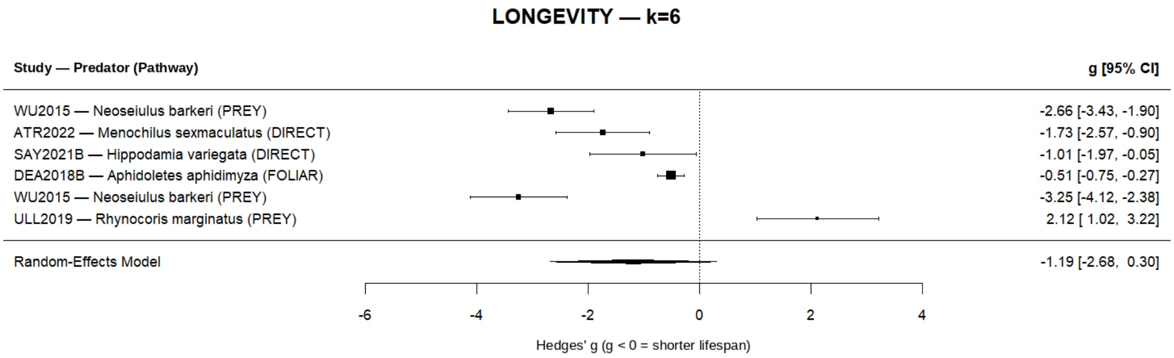
Supplementary Figure S2. Forest plot of survival effects (log OR) of EPF on predatory arthropods (k = 31 observations). OR > 1 = lower survival in EPF-treated group (pooled OR = 7.665, 95% CI: 4.322–13.593, p < 0.001).



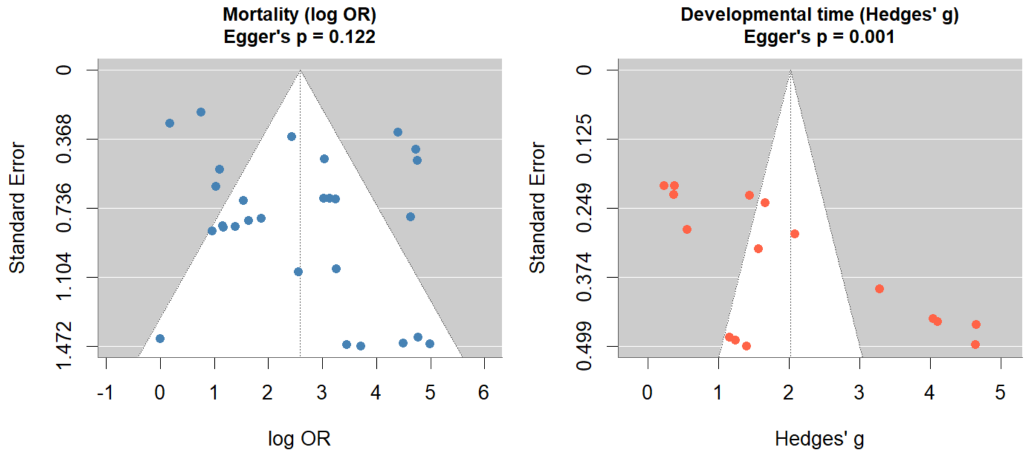
Supplementary Figure S3. Forest plot of developmental time effects (Hedges' g) of EPF on predatory arthropods (k = 16 observations). g > 0 = prolonged development in EPF-treated group (revised pooled g = 2.02, 95% CI: 1.26–2.78, p < 0.001).



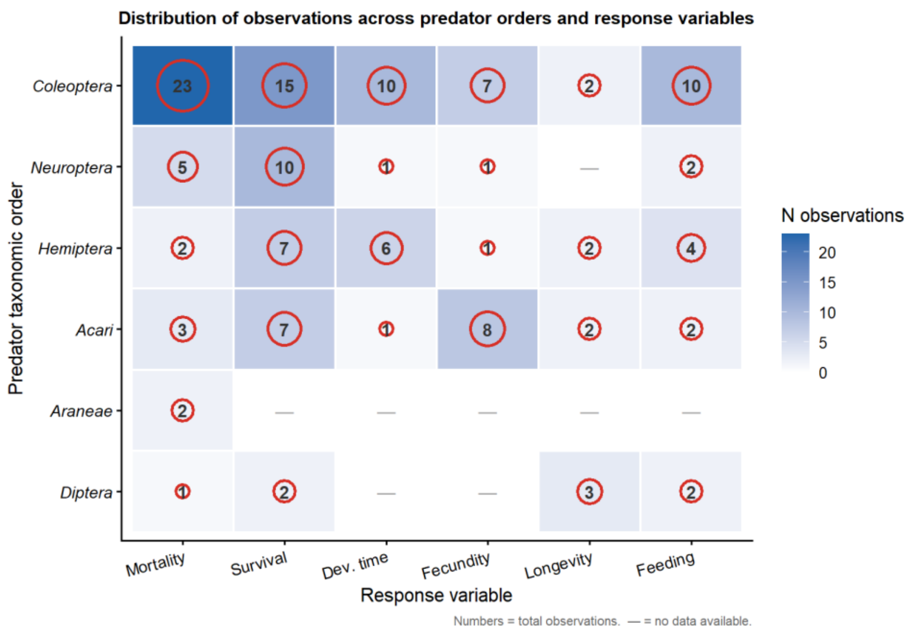
Supplementary Figure S4. Forest plot of fecundity effects (Hedges' g) of EPF on predatory arthropods (k = 10 observations). g < 0 = reduced fecundity (pooled g = −0.86, 95% CI: −1.33 to −0.39, p < 0.001).



Supplementary Figure S5. Forest plot of longevity effects (Hedges' g) of EPF on predatory arthropods (k = 6 observations). g < 0 = shorter lifespan. Pooled g = −1.189 (p = 0.119, not significant). Note conflicting effect directions across studies.



Supplementary Figure S6. Funnel plots for publication bias assessment of mortality (log OR, left) and developmental time (Hedges' g, right). Egger's regression test detected no significant funnel asymmetry for mortality (z = 1.60, p = 0.122), whereas developmental time showed significant asymmetry (z = 4.06, p = 0.001). Egger's test was also applied to the remaining variables (funnel plots not shown): survival was asymmetric (z = 4.79, p < 0.001) and fecundity was symmetric (z = 0.68, p = 0.517); longevity (k = 6) was not tested owing to insufficient power.



Supplementary Figure S7. Distribution of observations across predator taxonomic orders and response variables. Colour intensity and bubble size indicate the number of observations. Blank cells indicate no available data, highlighting priority areas for future research.