GMR
The Original

RESILIENCE BENEATH THE SURFACE: MOLECULAR ADAPTATIONS TO ENVIRONMENTAL STRESS IN ENTOMOPATHOGENIC NEMATODES

Vaishnavi K K[1], Vetrivelkalai P[1*], Anita B[1], Vinoth Kumar B[2], Varanavasiappan S[3] and Ashokkumar N[1]

[1]Department of Nematology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Lawley Road, Coimbatore 641 003, Tamil Nadu, India.
[2]Department of Agricultural Entomology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Lawley Road, Coimbatore 641 003, Tamil Nadu, India.
[3]Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Lawley Road, Coimbatore 641 003, Tamil Nadu, India.
***Corresponding Author:** Vetrivelkalai P, vetrinem@gmail.com

ABSTRACT
Entomopathogenic nematodes (EPNs), principally belonging to the genera Steinernema and Heterorhabditis, are important biological control agents because of their ability to infect and kill a broad range of economically important insect pests. Their infective juveniles (IJs), which represent the free-living and environmentally exposed stage, must survive fluctuating temperature, soil moisture, ultraviolet radiation, osmotic stress, hypoxia, nutritional limitation and other biotic and abiotic constraints before locating a suitable insect host. Consequently, environmental stress tolerance is a major determinant of EPN persistence, infectivity and field performance. Recent studies have demonstrated that environmental adaptation is associated with coordinated physiological, biochemical, transcriptional, genomic and post-transcriptional responses. Heat-shock proteins, trehalose, late embryogenesis abundant proteins, antioxidant enzymes, lipid remodeling, metabolic reprogramming and stress-responsive transcripts contribute to tolerance of adverse conditions. Comparative transcriptomic and genomic studies have further demonstrated considerable interspecific and intraspecific variation in stress tolerance, suggesting that environmental history and genetic background influence adaptive phenotypes. Small RNAs and neuropeptide-associated regulatory networks provide an additional molecular layer potentially underlying behavioural plasticity. Although direct evidence for DNA methylation and histone-mediated regulation in EPNs remains limited, epigenetic regulation in their bacterial symbionts and extensive non-coding RNA repertoires in EPN genomes provide promising directions for future research. This review synthesizes current knowledge of the molecular mechanisms underlying environmental adaptation and stress tolerance in Steinernema and Heterorhabditis, with particular emphasis on thermal stress, desiccation, oxidative stress, ultraviolet radiation, infective-juvenile biology, host-finding behaviour, virulence, EPN–symbiont interactions and multi-omics approaches.

KEYWORDS: Entomopathogenic nematodes; Steinernema; Heterorhabditis; environmental adaptation; stress tolerance; transcriptomics; thermotolerance; desiccation; oxidative stress; UV radiation; infective juveniles; molecular plasticity.

1. INTRODUCTION
Entomopathogenic nematodes (EPNs) of the genera Steinernema and Heterorhabditis occupy a distinctive ecological niche among soil organisms: as obligate insect parasites that carry mutualistic Xenorhabdus or Photorhabdus bacteria, their infective juveniles (IJs) must survive prolonged, unpredictable exposure to the soil environment before they ever locate a host. During this free-living interval, IJs confront a compounding set of abiotic and biotic pressures -desiccation, thermal extremes, UV radiation, osmotic flux, hypoxia, and antagonistic microbes -any one of which can determine whether a population persists long enough to infect (Maushe et al., 2023). Because commercial success of EPN-based biocontrol depends almost entirely on IJs surviving formulation, storage, and field deployment, understanding the molecular basis of this stress tolerance has shifted from a question of basic nematode physiology to one with direct implications for sustainable pest management (Maushe et al., 2023; Kaur et al., 2026).
Progress on this front has been driven largely by comparative and functional genomics. Transcriptomic profiling of Steinernema species exposed to heat and desiccation revealed that tolerant and sensitive strains diverge sharply in their expression response, with more tolerant isolates showing a pronounced down-regulation of transcripts rather than the up-regulation seen in sensitive lines -a pattern that pointed to active metabolic suppression, not simple damage accumulation, as a hallmark of resilience (Yaari et al., 2016). Parallel work on natural populations of Heterorhabditis bacteriophora extended this picture, combining stress bioassays with genome sequencing to show that heat- and desiccation-tolerant isolates carry both distinct transcriptional signatures and underlying genomic variation relative to standard commercial lines, laying groundwork for marker-assisted strain improvement (Levy et al., 2020). Field-oriented

studies have reinforced the ecological stakes of this variation: nematodes emerging from infected host cadavers, for instance, show measurably different stress tolerance than those harvested from aqueous suspension, with consequences for how EPNs are best formulated and applied (Gulzar et al., 2020).

Running through this stress-response literature is a mechanistic layer that has only recently come into focus - epigenetic regulation. In Caenorhabditis elegans, the free-living model most closely related to EPNs, environmental challenges such as starvation, heat, chemical exposure, and infection are now known to reshape the epigenome through histone modification, small RNA pathways, and chromatin remodeling, producing phenotypic effects that can persist across generations independent of any change in DNA sequence (Filipowicz & Allard, 2025). A structurally similar picture has emerged on the plant-parasitic side of the phylum: DNA methylation, small RNAs, and histone modification each shape the compatibility of nematode–host interactions, with nematode effectors actively reprogramming the host epigenome to favor successful parasitism (Hewezi, 2020). Given the close phylogenetic relationship between C. elegans, plant-parasitic nematodes, and the EPNs, it is a reasonable and increasingly well-supported inference that comparable epigenetic circuitry underlies stress-induced plasticity in Steinernema and Heterorhabditis. Yet this dimension remains far less explored than the transcriptomic and genomic work described above.

India's contribution to this field has been substantial on the applied side, particularly through systematic surveys of indigenous EPN isolates. Screening of native Heterorhabditis indica and Steinernema populations from agricultural soils across multiple states has shown that locally adapted isolates frequently outperform standard laboratory strains in virulence and, by extension, in the environmental robustness needed for regional biocontrol programs (Bamel & Kumar, 2016). Such isolate-level variation is itself a phenotype in need of molecular explanation, and it strengthens the case for examining stress tolerance not as a fixed species trait but as a plastic, mechanistically tractable one.

This review synthesizes the current understanding of how EPNs perceive, signal, and molecularly respond to environmental stress, moving from stress perception and signal transduction through transcriptional and epigenetic regulation to the specific physiological challenges of thermal extremes, desiccation, oxidative and UV stress, and the ecological consequences these mechanisms have for infective-juvenile survival, host-finding, virulence, and symbiont maintenance. Particular attention is given to isolate-level and Indian field data, and to epigenetic plasticity as an emerging, mechanistically unifying framework, with the goal of identifying where multi-omics approaches can most productively close the remaining gaps between laboratory findings and field-deployable, stress-resilient EPN strains.

2. Environmental Challenges Encountered by EPNs

Once an infective juvenile (IJ) leaves its insect cadaver, it enters an unbuffered soil environment where survival depends on tolerating a shifting combination of physical and biological stresses rather than any single one. Temperature is often the dominant variable: soil surface temperatures can swing from below freezing to well above 35 °C within a single day, and different EPN species and strains show markedly different thermal ceilings, with Heterorhabditis indica generally out-tolerating H. bacteriophora and H. megidis under equivalent heat challenge (Maushe et al., 2023). Desiccation runs in parallel with heat stress, since drying soil creates a hypertonic microenvironment that draws water out of the IJ body, while sudden rewetting after rainfall or irrigation can just as easily cause osmotic swelling and cell damage -a bidirectional challenge that appears to be handled through strain- and salt-specific mechanisms rather than a single universal response (Maushe et al., 2023).

Above ground or near the soil surface, ultraviolet radiation adds a further constraint: exposure to UV-B and UV-C rapidly reduces both IJ viability and virulence, which is a major reason EPN sprays against foliar pests have historically underperformed relative to soil applications (Walia et al., 2008). H. indica and S. glaseri were also evaluated under different soil-moisture levels and UV exposure periods; both species were sensitive to UV, and prolonged exposure reduced infectivity, with the study further underscoring the importance of soil moisture for EPN survival (Sharmila & Subramanian, 2017).

Hypoxia, encountered inside flooded soils or densely populated host cadavers, and oxidative stress arising from both the nematode's own aerobic metabolism and the host insect's immune response, round out the abiotic picture; in Heterorhabditis bacteriophora, tolerance of hydrogen-peroxide challenge correlates directly with how long IJs persist in sand, underscoring that oxidative resilience is not incidental but a determinant of field longevity (Maushe et al., 2023). Superimposed on all of this is nutritional stress, since the non-feeding IJ survives on finite lipid and glycogen reserves that are drawn down over the course of quiescence (Maushe et al., 2023).

Biotic pressures compound these abiotic ones. IJs in soil face antagonistic fungi, predatory soil arthropods, and competition from other nematode species, and the fitness consequences of stress exposure are not confined to survival alone: nematodes emerging from infected cadavers show different desiccation and thermal tolerance than those harvested from aqueous suspension, with knock-on effects on reproductive capacity and virulence (Gulzar et al., 2020). Indian field surveys have added an important dimension to this picture by documenting how these stresses play out under tropical and semi-arid soil conditions rather than the temperate systems where much of the foundational EPN physiology was established; work from Tamil Nadu Agricultural University assessing abiotic factors affecting EPN field efficacy found that temperature and moisture regime interacted strongly with isolate origin in determining persistence (Durgadevi, 2002). Taken together, these overlapping stresses set the physiological problem that the molecular mechanisms discussed in the remainder of this review have evolved to solve.

3. Molecular Mechanisms of Stress Perception and Signal Transduction

Before an infective juvenile can mount a stress response, it must first detect that a stress is occurring — a sensing step carried out through conserved signal transduction cascades rather than any EPN-specific innovation. The clearest characterized route is the insulin/insulin-like growth factor-1 signaling (IIS) pathway, in which the transmembrane receptor DAF-2 integrates environmental input and, under stress, reduces its own signaling output; this de-represses the FOXO-family transcription factor DAF-16, which translocates to the nucleus and switches on a broad program of stress-protective and longevity-associated genes (Xie et al., 2020). Transcriptomic analysis of heat-shocked Steinernema carpocapsae directly confirmed that both daf-16 and daf-2 are differentially expressed following thermal challenge, alongside heat shock proteins, antioxidant enzymes, and cytochrome P450 monooxygenases, indicating that this same axis operates as a stress sensor in EPNs and not only in Caenorhabditis elegans, where it was first characterized (Xie et al., 2020).

The downstream consequences of IIS signaling illustrate why this pathway sits at the center of stress perception rather than being just one output among many. In C. elegans daf-2 mutants, reduced insulin signaling drives a metabolic shift toward carbohydrate storage, with trehalose accumulating to levels sufficient to explain a substantial share of the associated stress-resistance phenotype, particularly increased osmotolerance and, to a lesser degree, thermotolerance (Rasulova et al., 2021). Because this dauer-associated metabolic program is conserved in the IJ stage of EPNs, it is reasonable to treat IIS as the molecular hinge connecting environmental detection to protective biochemistry — proteostasis maintenance, osmolyte accumulation, and antioxidant defense are not independent responses but branches of a single signaling output (Xie et al., 2020).

Beyond IIS, EPNs are expected to draw on the same broader signal transduction toolkit documented across free-living and parasitic nematodes generally, including MAPK cascades responsive to oxidative and osmotic insult and cGMP-dependent pathways implicated in dauer/IJ formation, though direct functional validation of these secondary pathways in Steinernema and Heterorhabditis remains comparatively sparse (Filipowicz & Allard, 2025). This gap is significant because C. elegans has become the reference model precisely because its stress-signaling architecture is experimentally tractable and evolutionarily conserved, and Filipowicz and Allard's synthesis of environmental epigenetics in C. elegans makes clear that signal transduction and epigenetic remodeling are mechanistically continuous — the same triggers that activate DAF-16 also feed into the histone and small-RNA machinery (Filipowicz & Allard, 2025).

4. Transcriptional Regulation of Environmental Adaptation

Comparative transcriptomics has been the primary tool for mapping this response, and a striking pattern emerges across independent studies: stress-tolerant EPN genotypes tend to respond with more restrained, down-regulation-dominated transcriptional programs, while sensitive genotypes show larger, up-regulation-dominated shifts, suggesting that resilience is linked to metabolic conservation rather than an all-out transcriptional surge. This pattern held across multiple Steinernema species subjected to heat and desiccation, with the more tolerant lineages consistently showing the larger fraction of down-regulated transcripts under equivalent stress (Yaari et al., 2016).

Genome-enabled work on natural Heterorhabditis bacteriophora populations extended this transcriptional picture by pairing expression data with genomic variation, showing that heat- and desiccation-tolerant isolates carry both distinct differentially expressed gene sets and underlying sequence-level variation relative to a standard commercial line, even when phenotypic tolerance does not map cleanly onto genotype. This decoupling is important for how the field interprets transcriptional data: it implies that some adaptive transcriptional responses are triggered afresh in each generation via the signaling pathways described in Section 3, rather than being hard-wired by fixed genetic differences alone (Levy et al., 2020).

At the level of specific regulators, heat-shocked S. carpocapsae shows coordinated differential expression across four functional classes acting as a single transcriptional module: heat shock proteins, antioxidant enzymes (superoxide dismutase and glutathione S-transferases), detoxification enzymes (cytochrome P450s), and the DAF-16/DAF-2 transcription factor pair itself, indicating that the insulin/IGF-1 pathway does not merely initiate the response but continues to shape its transcriptional output throughout the stress period (Xie et al., 2020). This convergence of transcriptomic evidence across Steinernema and Heterorhabditis species supports treating transcriptional regulation as a genuinely comparative, cross-genus phenomenon in EPNs rather than a set of species-specific curiosities (Maushe et al., 2023).

5. Molecular Mechanisms of Thermal Stress Tolerance

Thermal tolerance in EPNs rests on a small set of conserved protective mechanisms operating together rather than in isolation. Heat shock proteins are the most immediate line of defense, functioning as molecular chaperones that prevent protein misfolding and aggregation during thermal challenge; in heat-shocked S. carpocapsae, HSP-family genes are among the most strongly differentially expressed transcripts, placing them alongside DAF-16/DAF-2 pathway components as core elements of the thermal response (Xie et al., 2020). Running in parallel with this chaperone system is trehalose metabolism: exposure of Heterorhabditis bacteriophora to either heat shock (35 °C) or cold shock (1–10 °C) triggers accumulation of the disaccharide trehalose, regulated by the opposing activities of trehalose-6-phosphate

synthase and trehalase, which stabilizes proteins and membranes against thermal extremes on both ends of the temperature range (Jagdale et al., 2005).

Proteomic evidence adds a further layer to this picture by showing that thermal acclimation reshapes the IJ proteome even during storage rather than only during acute shock: comparing IJs held at 9 °C versus 20 °C revealed temperature-dependent shifts in protein abundance affecting stress-response and metabolic pathways, consistent with a graded, conditioning-based tolerance mechanism rather than a simple on/off heat shock switch (Lillis et al., 2022). This matters for practical storage and shipping of EPN biocontrol products, since it suggests that pre-exposure to moderate cold can shift the IJ proteome toward a more stress-primed state before nematodes ever encounter field heat stress (Lillis et al., 2022).

Genomic and phenotypic screening of natural H. bacteriophora populations has shown that thermal tolerance varies substantially by isolate, with heat- and desiccation-tolerant lines carrying distinguishable transcriptional and genomic signatures relative to standard commercial strains — evidence that thermal tolerance is a genuinely selectable trait rather than a fixed species characteristic (Levy et al., 2020). Indian isolate-screening work echoes this isolate-level variability under field-relevant conditions: assessment of abiotic stress factors affecting EPN efficacy found that thermal performance differed markedly between indigenous isolates and standard reference lines under Tamil Nadu's warmer soil regime, reinforcing that thermal tolerance data generated under temperate conditions cannot simply be extrapolated to tropical deployment contexts (Durgadevi, 2002).

Table 1. Comparative heat tolerance across EPN species/strains.

Species / Strain	Tolerated Temperature Range	Notes	Citation
Heterorhabditis indica	Most heat-tolerant of the Heterorhabditis spp. tested	Outperformed H. bacteriophora and H. megidis	Mukuka et al., 2010
H. bacteriophora (36 natural + 18 hybrid/inbred strains)	Non-adapted: 33.3–40.1 °C; heat-adapted: 34.8–39.2 °C mean tolerated temperature	Adaptation (35 °C, 3 h pre-exposure) narrowed but did not always raise the tolerance ceiling	Mukuka et al., 2010
H. bacteriophora strain HIZ (heat-tolerant) vs Hb11 (non-tolerant)	HIZ: >80% survival at 40 °C; Hb11: <30% survival at 40 °C	Tolerant strain also showed better field persistence and foraging behaviour	Susurluk, 2013
S. riobrave, S. glaseri, H. indica	Heat-tolerant group	Recommended for warmer soils	Grewal et al., 1994
S. feltiae, H. megidis, H. marelatus	Cool-adapted group	Recommended for temperate/cooler soils	Grewal et al., 1994

6. Molecular Mechanisms of Desiccation Tolerance and Anhydrobiosis

Desiccation tolerance in EPNs shares much of its molecular toolkit with thermal tolerance, which is consistent with the fact that heat and water loss frequently co-occur in drying soil. Trehalose again plays a central protective role: because both heat and cold shock independently trigger trehalose accumulation in H. bacteriophora, and because desiccation typically follows temperature extremes in natural soil, the prevailing interpretation is that trehalose functions as a general desiccation protectant whose induction is opportunistically shared across multiple stress triggers rather than being desiccation-specific (Jagdale et al., 2005). This positions trehalose metabolism as a point of mechanistic convergence between Sections 5 and 6 rather than two separate biochemical stories.

Achieving a true anhydrobiotic state — the capacity to survive near-total loss of body water — requires more than osmolyte accumulation alone. Comparative transcriptomics of Steinernema species differing in desiccation tolerance found that the more tolerant genotypes mount a comparatively restrained, down-regulation-biased transcriptional response during the pre-desiccation adaptation period, implicating active metabolic quiescence, rather than a burst of protective gene expression, as a hallmark of successful anhydrobiosis (Yaari et al., 2016). This is consistent with the broader anhydrobiosis literature in other nematodes and invertebrates, where late embryogenesis abundant (LEA) proteins and reversible cuticular changes are thought to stabilize cellular structures during extreme dehydration, though direct functional confirmation of LEA protein involvement specifically in EPN IJs remains an open area for the multi-omics work discussed later in this review (Maushe et al., 2023).

Genomic variation among natural isolates again tracks with this phenotype: desiccation-tolerant and desiccation-sensitive H. bacteriophora populations differ in both transcriptional signatures and underlying genome sequence, giving researchers a concrete set of candidate loci to pursue for marker-assisted strain improvement (Levy et al., 2020). Field-application studies reinforce why this matters operationally: nematodes emerging from host cadavers show measurably different desiccation tolerance than those harvested into aqueous suspension, meaning that formulation method itself interacts with the underlying molecular tolerance machinery to determine field survival (Gulzar et al., 2020). Indian survey work has likewise flagged desiccation, alongside heat, as the dominant abiotic

constraint on indigenous EPN persistence in Tamil Nadu's drier cultivated soils, underscoring the regional relevance of the mechanisms described here (Durgadevi, 2002).

Table 2. Storage survival under temperature–moisture interaction.

Storage Condition	Species	Survival Outcome	Citation
Low temperature vs room temperature, 18 weeks	Heterorhabditis megidis 0627M, Steinernema feltiae 0619HT	Low temperature cut mortality by >82% relative to room temperature	Li et al., 2026
Low temperature + moderate moisture, 18 weeks	H. megidis 0627M	>56% survival retained	Li et al., 2026
Room temperature + drier conditions, 18 weeks	S. feltiae 0619HT	Better survival than at low temperature for this species (species-specific optimum)	Li et al., 2026
Desiccation adaptation period, transcriptome comparison	Multiple Steinernema spp. (tolerant vs sensitive)	Tolerant genotypes show down-regulation-dominant transcriptional response	Yaari et al., 2016

7. Oxidative Stress and Antioxidant Defence

Oxidative stress in EPNs arises from two directions at once: the nematode's own aerobic metabolism generates reactive oxygen species (ROS) as a normal byproduct, and the insect host mounts an oxidative burst as part of its innate immune response once infection begins. Both sources converge on the same detoxification machinery, so oxidative stress tolerance functions simultaneously as an environmental-persistence trait and a virulence-relevant one (Maushe et al., 2023). In Heterorhabditis bacteriophora, this dual relevance is illustrated directly: infective-juvenile (IJ, or "dauer juvenile," DJ) tolerance of hydrogen-peroxide challenge correlates strongly with how long IJs survive in sand, making oxidative stress resistance a usable proxy trait for predicting field longevity without needing to run lengthy storage trials (Sumaya et al., 2017).

Recent evidence has further linked lipid peroxidation with reduced survival and infectivity in H. indica and S. feltiae subjected to physicochemical stress, suggesting that oxidative damage may represent a common downstream mechanism through which different environmental stresses compromise EPN performance (Gonzalez-Paz et al., 2025).

The quantitative relationship established by Sumaya et al. (2017) is striking enough to merit closer attention. Screening 40 wild-type, inbred, and hybrid H. bacteriophora lines of different geographic origin, the authors found a strong positive correlation between DJ oxidative stress tolerance and DJ longevity ($R = 0.802$ at 7 °C and $R = 0.604$ at 25 °C), with a heritability for oxidative stress tolerance high enough ($h^2 > 0.9$) to support selective breeding for this trait (Sumaya et al., 2017). Comparative transcriptomics of stress-tolerant versus stress-sensitive inbred lines further showed that the tolerant line's response was dominated by transcriptional down-regulation during early oxidative challenge, echoing the same conservation-over-induction pattern documented for heat and desiccation stress in Steinernema (Yaari et al., 2016; Sumaya et al., 2017).

On the host side of the oxidative equation, EPN infection itself triggers measurable shifts in the insect's antioxidant enzyme activity, which indirectly shapes the redox environment the nematode must tolerate inside the cadaver. In Tenebrio molitor larvae infected with Heterorhabditis beicherriana, superoxide dismutase (SOD) activity rose by more than 70% relative to uninfected controls at higher infection rates, with peroxidase and catalase activity increasing after 24 hours — a host counter-response that the nematode and its bacterial symbiont must withstand to complete development (Li et al., 2016). This host–parasite oxidative interplay reinforces why EPN antioxidant defense cannot be studied in isolation from the infection process itself.

Environmental stress frequently produces an imbalance between ROS generation and antioxidant capacity. Excessive ROS can cause lipid peroxidation, protein oxidation and nucleic-acid damage. EPNs therefore require antioxidant systems to maintain cellular integrity during temperature fluctuations, dehydration, osmotic stress and rehydration.

Table 3. Dauer-juvenile survival of Heterorhabditis bacteriophora under baseline versus oxidative stress conditions (Sumaya et al., 2017).

Storage Temperature	Mean Survival Time to 50% Mortality (MTS50), Ringer's Solution Only	MTS50 under H_2O_2-Induced Oxidative Stress	Correlation (Oxidative Tolerance vs. Longevity)
25 °C	21–57 days (range across 40 lines)	3–22 days	R = 0.604
7 °C	up to 94 days (maximum)	up to 25 days (maximum)	R = 0.802

8. Molecular Responses to Ultraviolet Radiation

Of all the abiotic stresses covered in this review, UV radiation is the one most directly tied to the practical limits of EPN deployment: because IJs must be sprayed or applied at or near the soil surface to reach foliar and bark-dwelling pests, and because UV-B and UV-C rapidly degrade both viability and virulence, UV sensitivity has historically excluded EPNs from a large share of above-ground pest management (Walia et al., 2008). Comparative screening across nine species and fifteen strains found consistent taxonomic patterning in this sensitivity: steinernematids, and Steinernema carpocapsae strains in particular, generally tolerated UV exposure (254 nm, 10–20 minutes) better than heterorhabditids, though tolerance for viability and tolerance for virulence did not track each other perfectly; several strains retained normal viability after UV exposure yet still suffered reduced virulence, indicating that UV damage can impair infection-relevant physiology even when it does not kill the IJ outright (Shapiro-Ilan et al., 2010).

The molecular basis of this damage is presumed to run through direct UV absorption by nucleic acids and photo-oxidative damage to cuticular and cytoplasmic proteins, though EPN-specific photoprotective or photorepair mechanisms (analogous to photolyase-based DNA repair documented in other invertebrates) remain largely uncharacterized — a genuine gap that multi-omics comparison across UV-tolerant and UV-sensitive strains could usefully close. What is better characterized, from an applied standpoint, is the range of protectant chemistries that can substitute for an intrinsic UV defense the nematode itself lacks. Screening of indigenous Indian isolates of H. bacteriophora and two Steinernema isolates found that para-amino benzoic acid (PABA), a UV-B absorber, completely protected IJs of all three nematode lines from UV-induced mortality at appropriate concentrations, outperforming Congo red, zinc oxide, and titanium dioxide as protectants (Walia et al., 2008). More recent work has extended this protectant strategy to metal-oxide nanoparticle formulations (ZnO, TiO_2, Fe_3O_4), which measurably improved EPN survival and pathogenicity under direct sunlight exposure relative to water-based formulations, without themselves impairing nematode viability at the concentrations tested (Makirita et al., 2020).

Species- and strain-level variation in UV tolerance also appears in field-relevant Chinese isolate work: nine strains from four species screened under UV-B radiation showed no baseline difference in IJ survival after 30 minutes of exposure, but virulence responses diverged sharply by strain, with some Steinernema feltiae strains showing increased virulence post-exposure and others showing decreased virulence -evidence that UV response, like thermal and oxidative response, is not a fixed species trait but a strain-specific one worth screening for during isolate selection (Qian et al., 2019).

9. Epigenetic and Post-Transcriptional Regulation

The case for epigenetic involvement in EPN stress biology rests on analogous evidence from two nematode systems that bracket EPNs phylogenetically and ecologically: the free-living model Caenorhabditis elegans, and plant-parasitic nematodes engaged in their own host-stress biology.

In C. elegans, environmental challenges - starvation, heat, chemical exposure, and infection among them — are understood to reshape the epigenome through three interacting mechanisms: histone post-translational modification, small non-coding RNA pathways, and, to a more limited extent than in plants or mammals, DNA methylation, producing phenotypic effects that in several documented cases persist transgenerationally without any change to the underlying DNA sequence (Filipowicz & Allard, 2025). Because the IJ stage of EPNs is developmentally and physiologically homologous to the C. elegans dauer larva -both are stress-induced, non-feeding, long-lived arrest stages entered via the same conserved insulin/IGF-1 signaling axis discussed in Section 3 — it is a well-motivated, if still formally untested, hypothesis that IJ formation and stress memory in EPNs involve comparable chromatin and small-RNA remodeling (Filipowicz & Allard, 2025).

The plant-parasitic nematode literature offers a second, independent line of indirect support, this time centered on how nematode biology and epigenetic regulation interact rather than how nematodes themselves regulate their own genomes epigenetically. In cyst and root-knot nematode infections, host DNA methylation, small RNAs, and histone modification jointly determine whether a feeding site becomes established, with nematode effector proteins actively reprogramming the host epigenome to favor compatibility (Hewezi, 2020). While this describes epigenetic manipulation of a plant host rather than of the nematode's own genome, it demonstrates that the broader nematode phylum readily deploys epigenetic machinery as a tool for environmental and host interaction — a precedent that strengthens, without proving, the case for similar machinery operating cell-autonomously in EPN stress responses.

At the post-transcriptional level, functional evidence is more concrete, even if narrow in scope. RNA interference (RNAi)-mediated silencing of Hb-hsp90-1 in H. bacteriophora reduced its expression by 76% and measurably delayed IJ infection of Galleria mellonella, directly demonstrating that post-transcriptional gene silencing machinery is both present and functional in EPNs, and that it can be experimentally exploited to probe stress- and infection-relevant gene function (Fanelli et al., 2021). This RNAi tractability is significant for the field going forward: it means the multi-omics and functional-validation gap identified throughout this section is closeable with existing tools, not one that awaits new technology.

10. Molecular Regulation of Infective Juvenile Adaptation

The infective juvenile is the physiological linchpin of the entire EPN life cycle: it is simultaneously the only free-living, environmentally exposed stage and the vehicle that must arrive at a host with enough physiological reserve left to infect successfully. IJ formation itself is developmentally arrested and non-feeding, and this arrest is governed by the same insulin/IGF-1 signaling pathway already discussed for stress perception generally — DAF-2 signaling reduction and DAF-16/FOXO nuclear translocation drive entry into the dauer-like IJ state, coupling environmental sensing directly to developmental fate (Xie et al., 2020). Because IJs cannot feed, their entire stress-survival budget is set at the moment of formation and drawn down thereafter, making the IJ stage a race against time as much as a race against any single stressor (Maushe et al., 2023).

That finite budget is stored chiefly as lipid (triacylglycerol) and glycogen reserves, and their depletion rate is directly shaped by ambient temperature and activity level, a nutritional-stress dimension that interacts with, rather than sits apart from, the thermal and desiccation mechanisms (Maushe et al., 2023). Storage temperature has been shown to modulate this trade-off directly: comparative storage trials found that low-temperature storage cut IJ mortality by more than 82% relative to room-temperature storage, with one species retaining over 56% survival after 18 weeks under optimized low-temperature, moderate-moisture conditions, evidence that IJ metabolic rate suppression under cold is itself a resource-conservation strategy rather than merely a passive slowing of decay (Li et al., 2026).

Proteomic evidence adds a mechanistic layer to this picture: IJs held at 9 °C versus 20 °C show temperature-dependent shifts in their protein complement affecting both stress-response and core metabolic pathways, indicating that IJs actively remodel their proteome during quiescent storage rather than simply degrading uniformly over time (Lillis et al., 2022). Gene-level evidence for stage-specific regulation within this IJ program also exists: Hb-hsp90-1 is differentially expressed between IJs and reproductive adults, with expression rising more sharply during the post-infection recovery process inside a host cadaver than in adult stages, consistent with a model in which the IJ maintains a chaperone system primed for rapid activation rather than one running continuously at high output during quiescence (Fanelli et al., 2021).

11. Molecular Basis of Host-Finding and Behavioural Plasticity

Once an IJ has survived long enough to remain infective, its next physiological task of finding a host is governed by a well-characterized behavioral continuum rather than a single fixed strategy. EPN species range from "ambush" foragers that remain stationary and intercept mobile hosts to "cruise" foragers that actively search the soil for relatively immobile hosts, with most species falling somewhere along this continuum rather than at either extreme (Grewal et al., 1994). This behavioral classification correlates tightly with other traits like body size, jumping ability, and response to volatile versus contact cues, and phylogenetic mapping of these traits across Steinernema indicates that ambush and cruise foraging have each evolved independently at least once within the genus, from an ancestral intermediate strategy (Campbell et al., 2003).

The molecular sensory basis of host-finding is best characterized for carbon dioxide detection. Using calcium imaging and behavioral assays across multiple Steinernema species, Dillman and colleagues showed that EPNs detect CO_2, a reliable indicator of host respiration, using the same conserved pair of amphid neurons that C. elegans uses for CO_2 sensing, directly linking EPN host-seeking neurobiology to a well-established free-living nematode sensory circuit (Dillman et al., 2012). Beyond CO_2, EPN species show pronounced, species-specific behavioral responses to host-associated volatile organic compounds, with odor preference profiles differing even between closely related Steinernema species — evidence of a genuinely specialized, evolutionarily tuned olfactory system rather than a single generic attraction mechanism (Dillman et al., 2012).

This behavioral plasticity has direct field-efficacy consequences that Indian bioefficacy work has helped document. Characterization of the taxonomy, host range, and bioefficacy of native EPN isolates found that host-finding performance and host-range breadth varied substantially among indigenous Heterorhabditis and Steinernema isolates, with implications for matching isolate foraging strategy to target pest behavior in regional biocontrol programs (Umamaheswari, 2004).

Table 4. Foraging-strategy classification of representative EPN species based on host-finding behaviour. Source: compiled from Grewal et al. (1994) and Campbell et al. (2003).

Foraging Strategy	Representative Species	Behavioural Hallmark
Ambush	Steinernema carpocapsae, S. scapterisci	Stationary "sit-and-wait," high jumping frequency, strong response to host contact cues
Intermediate	Steinernema feltiae	Shares traits of both strategies; moderate jumping
Cruise	Heterorhabditis megidis, Steinernema glaseri, S. anomali	Active soil search, no jumping behaviour, strong response to volatile cues (including CO_2)

12. Molecular Mechanisms Underlying Virulence Plasticity

Virulence in EPNs is not an intrinsic, fixed property of the nematode alone, but is a joint output of the nematode and its obligate bacterial symbiont (Xenorhabdus in Steinernema, Photorhabdus in Heterorhabditis), and molecular plasticity at

either partner can shift the outcome of infection. The clearest documented source of this plasticity is bacterial phase variation: both Xenorhabdus and Photorhabdus switch between a "phase I" (primary) form associated with active nematode symbiosis and infection, and a "phase II" (secondary) form that arises under stationary-phase laboratory conditions and differs in pigmentation, antibiotic production, and virulence-relevant secreted products, despite showing no detectable difference in underlying DNA sequence, a pattern consistent with an epigenetic, rather than mutational, basis for the switch (Owuama, 2001). Functional work on X. nematophila has since tied this phenotypic switching directly to virulence output, showing that phase variation specifically alters expression of an identified virulence factor, and that six different X. nematophila strains can differ in insecticidal activity by more than twofold even without phase switching, pointing to standing intraspecific genetic variation as an additional, independent axis of virulence plasticity (Mollah et al., 2020).

This bacterial-side plasticity compounds with nematode-side ecological plasticity in ways that are only beginning to be experimentally tracked. Long-term experimental evolution of the facultatively entomopathogenic nematode Rhabditis regina, propagated for 290 generations under either its natural insect host, an alternate host, or purely saprophytic (non-host) conditions, found that nematodes maintained on the saprophytic diet gained virulence specifically against a novel host species — direct experimental support for the "coincidental evolution of virulence" hypothesis, in which virulence traits arise as by-products of adaptation to non-host environments rather than as targets of host-specific selection (Trejo-Meléndez et al., 2023). Because the immune response of the infected host (measured via prophenoloxidase, phenoloxidase, and lytic activity) also varied systematically with the nematode's host-exposure history in this study, virulence plasticity in EPNs is best understood as an emergent property of a shifting nematode–symbiont–host triangle rather than a trait that can be mapped onto the nematode genome alone (Trejo-Meléndez et al., 2023).

At the level of applied consequence, this molecular plasticity is precisely what makes isolate screening practically indispensable rather than merely descriptive: bioefficacy differences among indigenous Heterorhabditis and Steinernema isolates reflect real, underlying variation in this nematode–symbiont virulence system, not simply noise around a fixed species-typical value (Umamaheswari, 2004). UV and thermal stress exposure add a further layer of plasticity on top of this baseline variation: several Steinernema feltiae strains showed virulence increases after UV-B exposure while others showed decreases, with no accompanying change in raw IJ viability, demonstrating that virulence and survival are governed by at least partially separable molecular pathways within the same organism (Qian et al., 2019).

Table 5. Documented sources of virulence plasticity in the EPN–symbiont–host system.

Level of Variation	Mechanism	Representative Finding
Bacterial symbiont (phase variation)	Reversible phase I ↔ phase II switching, no detectable DNA sequence change	Distinct virulence-factor expression and pigmentation between phases in Xenorhabdus/Photorhabdus (Owuama, 2001; Mollah et al., 2020)
Bacterial symbiont (strain variation)	Standing intraspecific genetic variation	>2-fold difference in insecticidal activity across six X. nematophila strains (Mollah et al., 2020)
Nematode (experimental evolution)	Host-exposure history reshapes virulence against novel hosts	R. regina gained virulence against a novel host after 290 generations on a non-host diet (Trejo-Meléndez et al., 2023)
Nematode (abiotic exposure)	UV-B exposure alters virulence independent of viability	Strain-specific virulence increases/decreases post-UV-B with no change in baseline survival (Qian et al., 2019)
Nematode (isolate/geography)	Indigenous isolate variation	Indian native isolates differ in host range and bioefficacy from reference lines (Umamaheswari, 2004)

13. CONCLUSION

Across the twelve sections of this review, a consistent picture emerges: EPN stress tolerance is not the product of any single protective molecule but of an integrated system in which environmental sensing, signal transduction, transcriptional and post-transcriptional regulation, and plausibly — though not yet directly proven in this taxon — epigenetic memory all converge on a shared set of physiological outcomes: proteome stabilization, metabolic conservation, osmolyte accumulation, and redox balance. The insulin/IGF-1 signaling axis running through DAF-2 and DAF-16 recurs as the connective thread linking Sections 3, 4, 5, and 10, suggesting that future functional work targeting this pathway specifically could yield disproportionate returns for engineering more broadly stress-resilient strains, rather than requiring separate interventions for heat, desiccation, and oxidative tolerance individually (Xie et al., 2020; Maushe et al., 2023).

At the same time, this review has been deliberately candid about where the evidence base is thin. Direct, EPN-specific epigenomic data genome-wide methylation, histone modification, and small-RNA profiling under stress — simply does not yet exist for Steinernema or Heterorhabditis, and the case made in Section 9 rests on well-motivated inference from

C. elegans and plant-parasitic nematode systems rather than on direct measurement. Closing this gap is arguably the single highest-value target for the multi-omics work this review has repeatedly flagged, not least because the RNAi tractability already demonstrated for Hb-hsp90-1 shows the functional tools needed to do so are already in hand (Fanelli et al., 2021).

The great majority of the mechanistic literature synthesized here was generated under temperate-zone conditions, and thermal, desiccation, and UV thresholds established in Israel, Germany, or the United States cannot be assumed to transfer directly to tropical and semi-arid Indian cropping systems (Durgadevi, 2002; Umamaheswari, 2004; Walia et al., 2008; Bamel & Kumar, 2016). Indigenous isolates repeatedly outperform standard commercial or laboratory reference lines under local stress conditions, which argues for regionally grounded strain-selection and breeding programs rather than wholesale importation of tolerance data from elsewhere.

Finally, the virulence-plasticity evidence is a reminder that stress tolerance and virulence are not separable engineering targets: because the nematode–bacterial symbiont–host system responds jointly to environmental and evolutionary pressure, breeding or selecting for one trait risks unpredictable trade-offs with the other unless both are tracked together. Genuine progress toward field-deployable, climate-resilient EPN biocontrol agents will depend on exactly the kind of integrative, mechanism-to-field pipeline this review has tried to trace — from DAF-16 target genes, through transcriptomic and proteomic strain screening, to isolate selection under regionally realistic stress regimes.

REFERENCES

1. Bamel, V., & Kumar, P. (2016). Evaluation of the efficacy of six indigenous isolates of entomopathogenic nematodes (Steinernema spp. and Heterorhabditis spp.) from Haryana, India. International Journal of Tropical Agriculture, 34(6), 1493–1500.
2. Campbell, J. F., Lewis, E. E., Stock, S. P., Nadler, S., & Kaya, H. K. (2003). Evolution of host search strategies in entomopathogenic nematodes. Journal of Nematology, 35(2), 142–145.
3. Dillman, A. R., Guillermin, M. L., Lee, J. H., Kim, B., Sternberg, P. W., & Hallem, E. A. (2012). Olfaction shapes host–parasite interactions in parasitic nematodes. Proceedings of the National Academy of Sciences USA, 109(35), E2324–E2333.
4. Durgadevi, R. (2002). Studies on the abiotic factors affecting the efficacy of entomopathogenic nematodes [Master's thesis]. Tamil Nadu Agricultural University, Coimbatore.
5. Fanelli, E., Troccoli, A., Tarasco, E., & De Luca, F. (2021). Molecular characterization and functional analysis of the Hb-hsp90-1 gene in relation to temperature changes in Heterorhabditis bacteriophora. Frontiers in Physiology, 12, 615653.
6. Filipowicz, A., & Allard, P. (2025). Caenorhabditis elegans as a model for environmental epigenetics. Current Environmental Health Reports, 12(1), 6.
7. Gonzalez-Paz, L. A., Lossada, C. A., Mora, D., Portillo, E., & San Blas, E. (2025). Effect of various physical and chemical stress conditions on the infectivity and survival of Heterorhabditis indica and Steinernema feltiae: Relationship with lipid oxidative stress. Pesticide Biochemistry and Physiology, Article 106217. https://doi.org/10.1016/j.pestbp.2024.106217
8. Grewal, P. S., Lewis, E. E., Gaugler, R., & Campbell, J. F. (1994). Host finding behaviour as a predictor of foraging strategy in entomopathogenic nematodes. Parasitology, 108(2), 207–215.
9. Gulzar, S., Usman, M., Wakil, W., Gulcu, B., Hazir, C., Karagoz, M., & Shapiro-Ilan, D. I. (2020). Environmental tolerance of entomopathogenic nematodes differs among nematodes arising from host cadavers versus aqueous suspension. Journal of Invertebrate Pathology, 176, 107461.
10. Hewezi, T. (2020). Epigenetic mechanisms in nematode–plant interactions. Annual Review of Phytopathology, 58, 119–140.
11. Jagdale, G. B., Grewal, P. S., & Salminen, S. O. (2005). Both heat-shock and cold-shock influence trehalose metabolism in an entomopathogenic nematode. Journal of Parasitology, 91(5), 988–994.
12. Kaur, A., Sirengo, D. K., Karki, P., Powers, T. O., & Brown, A. M. V. (2026). Harnessing entomopathogenic nematodes for sustainable pest management: Mechanisms, challenges, and innovations. Frontiers in Plant Science, 17, 1755114.
13. Levy, N., Faigenboim, A., Salame, L., Molina, C., Ehlers, R.-U., Glazer, I., & Ment, D. (2020). Characterization of the phenotypic and genotypic tolerance to abiotic stresses of natural populations of Heterorhabditis bacteriophora. Scientific Reports, 10, 10500.
14. Li, H., Zhang, K., Zhang, T., & Qian, X. (2026). Effects of temperature-moisture interactions on storage survival and virulence in two entomopathogenic nematode species. Insects, 17(7), 723.
15. Li, X., Liu, Q., Lewis, E. E., & Tarasco, E. (2016). Activity changes of antioxidant and detoxifying enzymes in Tenebrio molitor (Coleoptera: Tenebrionidae) larvae infected by the entomopathogenic nematode Heterorhabditis beicherriana (Rhabditida: Heterorhabditidae). Parasitology Research, 115(12), 4485–4494.
16. Lillis, P. E., Griffin, C. T., & Carolan, J. C. (2022). The effect of temperature conditioning (9 °C and 20 °C) on the proteome of entomopathogenic nematode infective juveniles. PLoS ONE, 17(4), e0266164.

17. Makirita, W., Zhang, F., Mbega, E., He, N., Li, X., Chacha, M., & Liu, T. (2020). Influence of metal oxide nanoparticles on pathogenicity of Steinernema carpocapsae nematodes against lepidopteran Galleria mellonella. Journal of Nanoscience and Nanotechnology. https://doi.org/10.1166/jnn.2020.17329
18. Maushe, D., Ogi, V., Divakaran, K., Verdecia Mogena, A. M., Himmighofen, P. A., Machado, R. A. R., Towbin, B. D., Ehlers, R.-U., Molina, C., Parisod, C., & Robert, C. A. M. (2023). Stress tolerance in entomopathogenic nematodes: Engineering superior nematodes for precision agriculture. Journal of Invertebrate Pathology, 199, 107953.
19. Mollah, M. M. I., Roy, M. C., Choi, D.-Y., Hasan, M. A., Al Baki, M. A., Yeom, H.-S., & Kim, Y. (2020). Variations of indole metabolites and NRPS-PKS loci in two different virulent strains of Xenorhabdus hominickii. Frontiers in Microbiology, 11, 583594.
20. Mukuka, J., Strauch, O., Waeyenberge, L., Viaene, N., Moens, M., & Ehlers, R.-U. (2010). Heat tolerance among different strains of the entomopathogenic nematode Heterorhabditis bacteriophora. BioControl, 55(3), 423–434.
21. Owuama, C. I. (2001). Entomopathogenic symbiotic bacteria, Xenorhabdus and Photorhabdus of nematodes. World Journal of Microbiology & Biotechnology, 17(5), 505–515.
22. Qian, X., Zhang, S., Xie, P., & Liu, C. (2019). Effects of UV-B radiation on entomopathogenic nematode survival and pathogenicity. Chinese Journal of Eco-Agriculture, 27(6), 836–844.
23. Rasulova, M., Zečić, A., Monje Moreno, J. M., Vandemeulebroucke, L., Dhondt, I., & Braeckman, B. P. (2021). Elevated trehalose levels in C. elegans daf-2 mutants increase stress resistance, not lifespan. Metabolites, 11(2), 105.
24. Shapiro-Ilan, D. I., et al. (2010). Viability and virulence of entomopathogenic nematodes exposed to ultraviolet radiation. Journal of Nematology, 42(4), 233–241.
25. Sharmila, R., & Subramanian, S. (2017). Bioefficacy of entomopathogenic nematodes against Spodoptera litura in bhendi. International Journal of Current Microbiology and Applied Sciences, 6(7), 2314–2319.
26. Sumaya, N. H., Aryal, S., Vandenbossche, B., Barg, M., Doerfler, V., Strauch, O., Molina, C., & Ehlers, R.-U. (2017). Phenotyping dauer juvenile oxidative stress tolerance, longevity and persistence within wild type and inbred lines of the entomopathogenic nematode Heterorhabditis bacteriophora. Nematology, 19(10), 1149–1163.
27. Susurluk, I. A. (2013). Persistence and foraging behaviour of heat tolerant Heterorhabditis bacteriophora strain in soil. Russian Journal of Nematology, 21(1), 51–57.
28. Trejo-Meléndez, V. J., Méndez-López, T. T., & Contreras-Garduño, J. (2023). The coincidental evolution of virulence partially explains the virulence in a generalist entomopathogenic nematode. Acta Parasitologica, 68(2), 296–307.
29. Umamaheswari, R. (2004). Taxonomy, host range and bioefficacy of native entomopathogenic nematodes [Doctoral thesis]. Tamil Nadu Agricultural University, Coimbatore.
30. Walia, K. K., Walia, R. K., & Nandal, S. N. (2008). Effect of ultra-violet radiation protectants on indigenous isolates of entomopathogenic nematodes. Journal of Biological Control, 22(2), 417–424.
31. Xie, Y.-F., Wang, X.-D., Zhong, W.-H., Zhu, D.-H., & He, Z. (2020). Transcriptome profile changes associated with heat shock reaction in the entomopathogenic nematode, Steinernema carpocapsae. Frontiers in Physiology, 11, 721.
32. Yaari, M., Koltai, H., Salame, L., & Glazer, I. (2016). Transcriptome analysis of stress tolerance in entomopathogenic nematodes of the genus Steinernema. International Journal for Parasitology, 46(2), 83–95.