



# Influence of Abiotic Factors on Efficacy of Entomopathogenic Nematodes

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## Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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## ABSTRACT

Entomopathogenic nematodes (EPNs) are potential biocontrol agents against many insect pests. The infective juveniles (IJs) dwell in soil ecosystem in a free-living state which is responsible for pest suppression under certain environmental condition. These IJs are faced with a wide range of environmental conditions during this stage. Abiotic factors such as temperature, moisture/relative humidity, soil texture and structure, ultraviolet radiation influence the survival, growth and development, and reproduction of entomopathogenic nematodes and ultimately their performance on bioefficacy against insect pests.

**Keywords:** Abiotic factors; biocontrol agents; bioefficacy; Entomopathogenic nematodes(EPNs); movement; reproduction; survival.

## 1. INTRODUCTION

Entomopathogenic nematodes (EPNs) in the genera *Steinernema* and *Heterorhabditis* are well known biocontrol agents against a wide range of

insect pests. The infective juveniles (IJs) are obligate parasites, dwelling in soil ecosystem. They are associated with a symbiotic bacteria, *Xenorhabdus* (*Steinernema* spp.) and *Photorhabdus* (*Heterorhabditis* spp.), and the

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nematode-bacterium complex together kills the target insect within 24-48 hrs. They are safe to environment and human, domestic animals and plants, and not inducer of insect resistance. Therefore they are considered as safe alternative to chemical insecticides. However, their efficacy greatly depends on their host range, host finding strategy, and above all they are susceptible to environmental factors. The soil has a highly complex physical, chemical and biotic environment that responds to external environment as well. The majorities of susceptible target pests is within the soil environment and are supposed to spend at least part of their life-cycle in the soil. Thus soil environment influences the EPNs population.

## 2. RESPONSE OF ENTOMOPATHOGENIC NEMATODES (EPNS) TO VARIOUS ABIOTIC FACTORS

### 2.1 Temperature

Different species and/or strains of EPNs have different optimum temperatures for survival, movement, reproduction and infection [1]. "At low temperature (< 10-15°C), their movement is restricted and at high temperature (>30-40°C) lethal to many species. IJs are non-feeding, depend on their lipid reserves, which vary (32-38%) in composition amongst EPN species. IJs or dauer larval stage has lower levels of metabolic activity than other stages of their life-cycle. The low temperature induces inactivity in IJs, due to the decreased metabolic activity. Extreme ambient temperatures can lead to desiccation, increased metabolic rate and as a result more use of lipid reserves by EPNs, which leads to decreased infectivity with shorter reproduction rate of such nematodes" [2]. "However, in the soil environment, IJs are generally buffered from temperature extremes depending on soil quality. Optimum temperature range for storage of IJs is 5-15°C. It has been observed that activity of steinernematid species is more at lower temperatures than heterorhabditid species. At below 9°C and above 20°C storage of *S.carpocapsae*, the proteins reserves (42-46%) decreased by metabolism, stress and cytoskeleton. Catalase and copper oxide dismutase, the stress enzymes increased in abundance after storage at low temperature. IJs may enhance their survival in colder conditions by reducing metabolic activities which produce these free radicals by damaging cells and increasing their stress response against them. Some *Steinernema* species are reported to

be well-adapted to warm temperatures of their original habitat, e.g., *S.riobrave* and *S.glaseri*" [3]. "Similarly, *H. bacteriophora* (IS-5) which was isolated from the Negev Desert exhibits heat tolerance due to the presence of heat shock proteins (HSP)" [4]. Shapiro *et al.* [5] improved "a heat tolerant hybrid strain from *H. bacteriophora* (IS-5) and *H. bacteriophora* (Hp88) using heat shock proteins (HSP) through genetic methods and the resulting hybrid strain could able to kill *G. mellonella* larvae at 32°C". In general, freezing temperatures can be tolerated better by the IJs than high temperatures (above 37°C) by using some mechanisms [6] like freeze-avoiding mechanism: The sheath of *H. zealandica* prevents the IJ from super-cooling (-32°C) [7]; *S. feltiae* and *H. bacteriophora* shows freezing tolerant mechanism [8], and cryoprotective dehydration mechanism [9]. Wright [10] observed that "reproductive rate of many EPNs at low temperature was correlated with the bacterial symbiont growth rate e.g. *H.megidis*, and *S.feltiae*". Tailliez *et al.* [11] observed that "some *Xenorhabdus* species may have become adapted to either tropical or temperate regions as influenced by their nematode host. The following Table.1 shows required temperature range for survival, reproduction and infectivity of EPNs and application during conditions within this range will result in better performance".

### 2.2 Moisture

Moisture or relative humidity level is the most important abiotic factor influencing EPN population behavior which may vary among nematode species and isolates in different soil types [31-32]. Moisture also influences aeration of soil. EPN require adequate soil moisture levels for their survival, movement and infectivity [33]. Under low moisture condition, some species viz., *Steinernema* spp. reduce their body surface area by lowering their rate of metabolism, the survival strategy is known as anhydrobiosis. *S.carpocapsae* and *S.glaseri* survival was good at low soil moisture of 2 and 4% respectively [3]. Gradual water removal from the infective juveniles gives them time to adapt to the water loss conditions and thereby nematodes are resistant to desiccation. Natural soils are such kind of soil that allows the IJs to persist longer time. IJs may survive in desiccating conditions inside the host cadaver until the favorable soil moisture situation arise [34]. In order to minimize desiccation, spraying should be done during the evening and using supplemental wetting before and after application by using irrigation or rainfall

that helps in recovery of nematode infectivity and virulence [38]. For movement nematodes need a water film in soil pore. In saturated soil, oxygen levels decrease and nematode movement is restricted [35, 36]. “Several studies indicate that soil moisture also influences the bioefficacy of EPNs. There must be a continuous film of water between nematode and target insect for infection to occur. Nematode infectivity is highest at moderate soil moistures (–10 to –100 kPa), and lower in wet (–1 kPa) and dry (–1000 kPa) soil” [37]. “Infectivity of *H. bacteriophora*, *S. carpocapsae*, *S. feltiae*, and *S. glaseri* increased with moderate soil moisture in sandy loam soil” [39]. Similarly, *H. bacteriophora* DI and *S. glaseri* KG [40] and *S. glaseri* NC and *S. carpocapsae* ALL [35] showed lower infectivity in low and high soil moistures. Studies have also shown that

*H. bacteriophora* is less susceptible on *Popillia japonica* 3rd instar as the season changes from rainfall to spring [41]. Under high humidity (RH) and moderate temperature, *S. virgalemense* is highly susceptible to female *Planococcus ficus* and mortality rate of *P. ficus* decreases with decreasing relative humidity levels [42]. At least 8-24 h of RH( > 90%) is necessary for infection in case of above ground pests. Both inoculative and inundative application of IJs will work properly in habitats with high relative humidity. EPN formulation also requires suboptimal moisture level. Nematode life span increases when the nematode enters in a state of partial anhydrobiosis. Irrigation or rainfall can rehydrates formulated nematodes and they resume function as virulent individuals, ready to be applied in the field.

**Table 1. Effect of temperature on survival, reproduction and infectivity of Entomopathogenic nematodes**

Entomopathogenic nematodes	Survival	Reproduction	Infectivity	References
<i>Steinernema glaseri</i>	15-30°C	28°C	15-35°C 10-32°C 0-25°C	Grewal et al [2], [3], [12]
<i>S. carpocapsae</i> <i>S. carpocapsae</i> (Pocheon) <i>S. carpocapsae</i> (PDBCEM 6.11)	9 - 20°C	20-24°C	10-32°C 24°C 5-25°C 25 -32°C	Grewal et al [2], [13-15]
<i>S. feltiae</i>	15°C	12-25°C 18°C	10-15°C 10°C	Hazir et al [1], [16-19]
<i>S. anomali</i> (= <i>S. arenarium</i> ),	Can tolerate sub-zero temperatures	23°C	23°C	Kakouli-Duarte [20-21]
<i>S. riobrave</i>	35 °C	20-35°C	10-39° C 29°C	Ali and Wharton [21-22]
<i>S. siamkayai</i> Thailand strain <i>S. siamkayai</i> Tiruchirappalli strain	25-30 °C	25-30°C 20-35°C	25-30°C 37.5°C	Tailliez et al [11] ,[23]
<i>S. scapterisci</i>	In warm climates	20-32 °C	30°C	Hazir et al [24]
<i>H. bacteriophora</i>	-19 -30°C	15 - 30°C	10°C- 35°C	Ali and Wharton [21] [25-26]
<i>H. zealandica</i>	15-30°C	15-30°C	15-30°C	De [27-28]
<i>H. megidis</i>	9- 20°C	15-25°C	15°C 29°C	Grewal et al [2-3] ,[29-30]
<i>H. merelatus</i>	Warm climate	29°C	29°C	Grewal et al [2-3],[30]

### 3. SOIL TEXTURE AND STRUCTURE

Texture of soil is decided by the proportion of sand, silt and clay inherited by it during its formation. Soil structure determines the number, size and distribution of pore spaces in soil crumbs. These pores are partly or wholly filled with soil moisture. Soil texture and structure can have various effects on EPNs. Movement [43], infectivity of IJs [44] and survival or persistence of IJ [45] are positively correlated with soil structure [37]. In general, nematode movement and survival is better in sandy soils, whereas survival rates are poor in clay soils because of reduced aeration in the small soil pores [24]. *H. bacteriophora*, *S. carpocapsae*, and *S. glaseri* survival and movement were more in sandy loam than silty clay loam soils. Similarly, bulk density of soil also influences survival and infectivity of nematodes e.g., survival of *H. bacteriophora* decreased, and survival of *S. glaseri* increased with bulk density. The infectivity of *S. carpocapsae* increased but did not vary for *H. bacteriophora* and *S. glaseri*. Establishment of EPNs may vary with species and isolate with different soil type. Koppenhofer *et al.*, [46] reported that “establishment of *S. scarabaei* was more in sandy loam soil compared to clay loam soil”. Campos-Herrera and Gutiérrez [47] observed that “infection behavior varied among 14 different populations of *S. feltiae* in the same soil texture”. Shelmith *et al.*, [48] observed that “survival of *S. kariii* was 22-67%, with the highest occurrence being in clay soil, moderate in sandy clay and clay loam and the lowest being in the sandy clay soil”. Campos-Herrera and Gutiérrez [49] observed that “virulence of the Rioja isolate of *S. feltiae* negatively affected mortality of *Spodoptera littoralis* larvae in heavy soils”. The proportion of organic matter (OM) content in the soil along with mineral component affects nematode movement as OM content of soil improves soil structure, lowering bulk density, increasing the available space through the distribution of aggregates and pore size, thus OM content and soil compaction may influence EPNs infection behavior [50], and persistence in the soil [51]. Therefore, knowledge about soil structure and texture requirement of an EPN species/isolate is critical to optimizing their performance as biological control agents in the field.

### 4. SALINITY

Soil salinity has an insignificant effect on IJs survival. EPNs appear to tolerate a wide range of salinities. Several *Heterorhabditis* species/strains

have been isolated from soils near the seashore; therefore seawater has no negative impact on the survival of IJs. However, tillage and herbicide applications suppress EPN numbers, the movement of EPNs is restricted and their ability to find and recognize their host is reduced. Bednarek [52] observed that application of mineral fertilizers had negative impact on EPN population density.  $\text{CaCl}_2$  and KCl had no effect on the survival, movement or penetration behaviour of *H. bacteriophora* but moderate concentrations of these salts may enhanced the virulence. Although seawater has no negative effect on IJs, high concentrations of NaCl,  $\text{CaCl}_2$ , and KCl inhibit the movement and infectivity of *S. glaseri* [40].

### 5. OXYGEN AND pH

Oxygen is also a limiting factor on EPN population behavior, low oxygen level that can occur in water-saturated soils, clay soils (because of small pores) and soils with high organic matter content. As far as EPNs as a formulated product is concerned, the presence of interstitial spaces in formulations is beneficial to such nematodes, as it supports gas exchange. Soil moisture and aeration affect the soil pH. IJs can survive in a wide range of pH. Soil pH between 4 and 8 did not have an adverse effect on IJ survival, but a pH of 10 or higher was harmful. Kung *et al.*, [53] observed in laboratory trials that pH 10 reduced steinernematid nematodes survival. *S. bicornutum* was found only in neutral soils, and *S. silvaticum* only in acidic environments [54].

### 6. UV RADIATION

UV radiation effect on the survival, virulence, or reproduction of EPNs. Shapiro-Ilan *et al.* [55] investigated “UV tolerance of nine different EPN species and fifteen strains for survival and virulence. Their results showed that there was a significant variation in UV tolerance among EPN strains and species. Steinernematids commonly displayed higher levels of UV tolerance compared with the heterorhabditids. Furthermore, it has been reported that shorter wavelengths (254 nm) had a negative effect on virulence compared to longer wavelengths (366 nm)”.

### 7. CONCLUSION

For successful utilization of EPNs, ecology of the target pest should match with the activity of IJs.

Therefore, a better understanding of ecological strategies of IJs for its efficiency in biological control in the local agro-climatic conditions is needed. However, nematode establishment in adverse environment is based on IJs behavioural and physiological adaptation. The impact of temperature or moisture on EPN efficacy can be changed, by choosing the right EPN strain and applying these nematodes at the right time of the year, or postponing application until temperatures or moisture are optimal for the specific EPN strain used. The biological study of EPNs is advancing rapidly. Isolation and description of new species and described species still need to be studied in detail for their potential use as biological control agent. Thus the selectivity and safety of these biocontrol agent by minimizing their effects on other biocontrol agents and will facilitate their incorporation into IPM programs and ultimately contribute to the chemical-free management of many kind of target insect pests.

## COMPETING INTERESTS

Author has declared that no competing interests exist.

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