Influence of abiotic factors on efficacy of Entomopathogenic nematodes

Abstract:

Entomopathogenic nematodes (EPNs) are potential biocontrol agents against many insect pests. Abiotic factors such as temperature, moisture, soil texture canaffect the survival,growth and development, andreproduction of entomopathogenic nematodes and ultimately their efficacy against insect pests.

Key words: Entomopathogenic nematodes(EPNs) abiotic factors, biocontrol agents, survival, movement, reproduction , bioefficacy.

Introduction

Entomopathogenic nematodes in the genera *Steinernema* and *Heterorhabditis* are well known biocontrol agents against a wide range of insect pests. However, their efficacy greatly depends on their host range, host finding strategy, and above all their tolerance of environmental factors.EPN live in soil for short or long durations.The soil has a highly complex physical, chemical and biotic environment that responds to external environment as well. Thussoil environment influences the entomopathogenic nematode population.

Response of Entomopathogenic nematodes to various abiotic factors

Temperature:

The optimum temperatures for survival, movement, infection, and reproduction of EPNs vary among species and strains (Hazir *et al.*, 2001). IJ are non feeding, relying for energy on their internal lipid reserves, which vary in composition amongst EPN species. Dauers and IJs have lower levels of metabolic activity than other stages of their life-cycle. At higher temperatures, the infective juveniles have increased metabolic activity and deplete their energy reserves, shortening their life span. The low temperature induce inactivity in infective juveniles, such inactivity is characterized by decreased enzymatic activity and mobility, both reducing metabolic expenditures.Extended exposure to temperature extremes (below 0^{o}C or above 40^{o}C) is lethal to most species of entomopathogenic nematodes. In the soil environment, infective juveniles are normally buffered from temperature extremes. Extreme ambient temperatures can lead to desiccation, increased metabolic rate and consequently more rapid use of energy reserves by EPNs, which leads to shorter generation times, decreased infectivity and lowered virulence of

such nematodes (Grewal *et al*., 1994). For storage, the best longevity of infective juveniles is between 5 and 15^{o}C. Temperature below 10-15^{o}C can restrict their mobility, while temperatures higher than 30-40^{o}C can inactivate them.It is generally known that steinernematid species are more active at lower temperatures than heterorhabditidspecies. The proteins which decreased in abundance to the greatest extent in *S.carpocapsae* after both 9°C and 20°C storage were those associated with metabolism, stress and the cytoskeleton. Oxidative phosphorylation produces the most energy of all metabolic processes, but it also produces detrimental reactive oxygen species. Free radicals damage cells and contribute towards aging. Catalase, the stress enzyme which breaks down hydrogen peroxide, and copper oxide dismutase were both 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 and increasing their stress response against them.However, some *Steinernema* species are well-adapted to warm temperatures.It has been observed that the original climatic locality of the nematodes correlated with their temperature range (Kung *et al.* 1991).The heat survival by IJs is related to the presence of heat shock proteins (HSP). *H. bacteriophora*(IS-5) which was isolated from the Negev Desert exhibits heat tolerance (Glazer, 2002). Shapiro *et al.* (1997) improved a heat tolerant hybrid strain from *H. bacteriophora*(IS-5) and *H. bacteriophora*(Hp88) using classical genetic methods. The hybrid strain killed *G. mellonella*larvae at 32°C. In general, freezing temperatures can be tolerated better by the IJs than high temperatures (above 37°C) (Shapiro-Ilan *et al.*, 2014). To survive at freezing temperatures, IJs use three mechanisms; (1) freeze-avoiding: The sheath of *H. zealandica*prevents the IJ from supercooling (-32°C) (Wharton and Surrey, 1994). (2) freezing tolerant (Brown and Gaugler, 1997), or (3) cryoprotective dehydration (Wharton *et al.*, 2003). *S. feltiae*and *H. bacteriophora*can exhibit freezing tolerant andcryoprotective dehydration.Wright (1992) reported that reproduction rate of many entomopathogenic nematodes at low temperature was related to the growth rate of the bacterial symbiont. Tailliez*et al.* (2006) suggested that some *Xenorhabdus*species may have become adapted to tropical or temperate regions and/or influenced by the optimal growth and development temperatures of their nematode host.

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

Entomopathogenic nematodes	Survival	Reproduction	Infectivity	References
Steinernema glaseri	15-30°C	28°C	15-35°C	Kung *et al*.,1991;

			10-32ºC 0-25°C	Grewal *et al*. 1994; Jagdale &Gordon, 1997
S.carpocapsae *S. carpocapsae* (Pocheon) *S. carpocapsae* (PDBCEM 6.11)	9 - 20°C	20-24°C	10-32ºC 24°C 5-25°C 25-32°C	Grewal *et al*., 1994, Saunders & Webster, 1999; Brown *et al*., 2002; Yul *et al*., 2002
S. feltiae	15°C	15-25°C	10-15°C 10^{o}C	Grewal *et al*.,1996; Hazir *et al*., 2001; Chen *et al*., 2003; Radova&Trnkova ,2010 Flores *et al*.,2021
S. anomali (= *S. arenarium*),	can tolerate sub-zero temperatures	23^{o}C	23^{o}C	Kakouli-Duarte & Hague,1999; Ali &Wharton, 2013
S. riobrave	35 ºC	20-35ºC	10-39º C 29°C	Cabanillas *et al*., 1994; Ali& Wharton, 2013
S. siamkayai Thailand strain *S. siamkayai*Tiruchirappalli strain	25-30 ºC	25-30ºC 20-35ºC	25-30ºC 37.5°C	Chongchitmate*et al*. 2005; Tailliez*et al.*,2006
S. scapterisci	In warm climates	20-32 ºC	30ºC	Hazir *et al*. 2003
H. bacteriophora	-19 -30°C	15 - 30ºC	10ºC- 35ºC	Mason &Hominick 1995; Ali & Wharton, 2013; Milstead ,1981
H. zealandica	15-30°C	15-30°C	15-30°C	Waal *et al*., 2018; Aryal *et al*., 2022
H. megidis	9- 20°C	15-25°C	15°C Equal to or higher than 29°C	Dziegielewska *et al*.,2023; Kung *et al*., 1991; Grewal *et al*., 1994; Berry *et al*., 1997
H. merelatus	Warm climate	Equal to or higher than 29°C	Equal to or higher than 29°C	Kung *et al*., 1991 Grewal *et al*., 1994, Berry *et al*., 1997

Moisture:

Moisture is the most important factor influencing EPN populations (Shapiro-Ilan *etal*., 2007; Campos-Herrera *et al*., 2016).EPNrequire adequate soil moisture levels for their survival, locomotion and infectivity, which may vary among nematode species and isolates in different

soil types (Glazer 2002).Moisture also influences aeration of soil.Some species develop survival strategies under water stress conditions, by reducing the body surface area exposed to the air and by lowering their rate of metabolism. This process, known as anhydrobiosis, allows the nematode to become resistant to desiccation. Anhydrobiosis can be reversed by wetting the soil, causing a recovery of nematode infectivity and virulence. Gradual water removal from the infective juveniles gives them time to adapt to the desiccating conditions. Thus, natural soils allow the infective juveniles to persist longer in dry soil.Infective juveniles may survive desiccating conditions by remaining inside the host cadaver until the soil moisture situation improves (Grewal 2000).Some species of the *Steinernema* have the ability to enter a state of anhydrobiosis when exposed to low moisture contents (Koppenhöfer*et al*., 1995).Nematodes need a water film in the interstitial spaces of soil for effective movement.When this water film becomes too thin (i.e. in dry soil) or the interstitial spaces become completely filled with water (i.e. in saturated soil),oxygen levels decrease and nematode movement is restricted (Stuart *et al*., 2015).Several studies indicate that soil moisture influence infectivity of entomopathogenic nematodes, Nematode infectivity is highest at moderate soil moistures (−10 to −100 kPa), and lower in wet (−1 kPa) and moderately dry (−1000 kPa) soil (Koppenhofer and Fuzy, 2007).*S.carpocapsae* and *S.glaseri* survival was good at low soil moisture of 2 and 4% respectively(Kung *et al*., 1991) In general, a decrease in infectivity as soil moisture decreases is that these nematodes have entering a state of anhydrobiosis, but it can be increased by rehydrating the soil using irrigation or rainfall (Alekseev *et al*., 2006).Grant and Villani (2003) showed that infectivity of *H. bacteriophora*, *S. carpocapsae*, *S. feltiae*, and *S. glaseri*increased with soil moisture in sandy loam soils. Similarly,*H. bacteriophora*DI and *S. glaseri* KG (Molyneux and Bedding 1984) and *S. glaseri*NC and *S. carpocapsae*ALL (Koppenhöfer*et al*.,1995) showed lower infectivity in extreme,low and high (near the saturation point) 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 (Paoli *et al*., 2017). Under high humidity and moderate temperature, *S. virgalemense* is highly susceptible to female *Planococcusficus* and mortality rate of*P.ficus* decreases with decreasing relative humidity levels (Platt *et al*., 2018).Suboptimal moisture levels can be beneficial whenformulating nematodes. Water activity in the formulation can gradually be reduced to a point wherethe nematode enters a state of partial anhydrobiosis where nematode's metabolism ceases which leads to a lowered use ofenergy

reserves, increasing the life span of thenematode under suboptimal moisture levels.Pre-application of water rehydrates formulatednematodes and they resume function as virulentindividuals, ready to be applied in the field.

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 onEPNs and they should be evaluated for different species. Movement (Kapranas*et al*., 2021), infectivity of IJs (Toledo *et al*., 2009)and persistence (Koppenhöfer and Fuzy, 2006) are positively correlated if the space among soil particles is similar or greater than the diameter of an IJ (Stuart *et al.*, 2015).In general, the most favorable soil type for nematode movement is sandy soils, whereas clay soils have poorest survival rates because of low oxygen levels in the small soil pores (Hazir *et al.*, 2003). Besides soil mineral component, the proportion of organic media in the soil affects nematode movement.*H. bacteriophora, S. carpocapsae,* and *S. glaseri*survival and movement was more in sandy loam than silty clay loam or loamy soils. While survival of *H. bacteriophora*decreased, survival of *S. glaseri*increased with bulk density. Furthermore, the bulk density increased the infectivity of *S. carpocapsae*, but did not vary for *H. bacteriophora*and *S. glaseri.* Koppenhofer *et al*., (1998) reported that establishment of *S. scarabaei* was more in sandy loam soil compared to clay loam soil. However, this may vary with EPNs species and isolate. Campos-Herrera and Gutiérrez (2014) observed that infection dynamics varied among 14 different populations of *S. feltiae* in the same soil texture. Shelmith*et al*., (2007) reported that survival of *S. karii* 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 (2009) observed that heavy soils negatively affected the virulence of the Rioja isolate of *S. feltiae* on larvae of *Spodoptera littoralis* as an increase content of clay from 5 to 14%, caused an increase in the LC90 (to kill 90% of larvae in two days) from 220 to 4,178 IJs/cm^2. Organic matter (OM) content of soil improves soil structure, loweringbulk density, increasing the available space throughthe distribution of aggregates and pore size, thusOM content and soil compaction may influenceEPNs infection patterns (Kapranas*et al*., 2017), andpersistence in the soil (Shapiro and Lewis, 2018). Therefore, knowledge about soil

requirements of an EPN species/isolate is critical to optimizing their performance as biological control agents in the field.

Salinity

Soil salinity has a negligible effect on infective juvenile survival even at salinity above the tolerance levels of most crop plants.Seawater has no negative effects on the survival of several *Heterorhabditis*species/strains as they have been frequently isolated from soils near the seashore.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 (1998) observed that mineral fertilizers negatively affected EPN population density. EPNs appear to tolerate a wide range of salinities. $CaCl_2$and KCI had no effect on the survival, penetration efficiency, or movement through a soil column for *H. bacteriophora*but moderate concentrations of these salts enhanced the virulence. Although seawater has no negative effect on IJs, high concentrations of NaCl, $CaCl_2$, and KCl inhibit the infectivity and movement of *S. glaseri*(Grant and Villani, 2003).

Oxygen and pH

Oxygen is also a limiting factor in water-saturated soils and soils with high organic matter content. Soil moisture and aeration affect the soil pH.Low oxygen level that can occur in water-saturated soils, clay soils (because of small pores) and soils with high organic matter content.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*., (1990) 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 (Kanga *et al.*, 2012). 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 .

UV radiation

The survival, virulence, or reproduction of EPNs can be affected by UV radiation. Shapiro-Ilan *et al.* (2015) 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. Steinernematidscommonly 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).

Conclusion

A better understanding of ecological strategies of native nematodes for its efficiency in biological control in the local agro-climatic conditions is needed. Therefore, a means to lower the impact of temperature or moisture on EPN efficacy as biological agents should be enforced, 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.Therefore isolation and description of new species and described species still need to be studied in detail for their potential use as biological control agent.

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