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# Entomopathogenic Nematodes as Bioindicators of Soil Health

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Author's contribution

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

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**Review Article** 

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### ABSTRACT

Soil health or soil fertility is an ecological characteristic of an active system with the presence of many living organisms that promote plant growth. Assessment of soil health is done by chemical, physical and biological indicators. As soil organisms are naturally present in soil, therefore biological indicators are important key for monitoring soil quality. Soils are increasingly subjected to deterioration in physico-chemical properties along with reduced biodiversity that leads to low productivity. Invertebrates present in soil remain directly in contact with soil and can serve as biological indicators. Among these, nematodes are important member to consider as biological indicator. An important group of nematodes are entomopathogenic nematodes (EPNs), which are widely used as biocontrol agent against various economically important insect pests. Some of the uniqueness and availability of EPNs makes them valuable candidates as soil health indicators. Land management practices affect the biological parameters of EPNs. This review illustrates the role of EPNs as bioindicator of soil health and their contribution to plant health as well as agricultural system.

Keywords: Soil health, bioindicator; entomopathogenic nematodes (EPNs); soil pollution; food productivity.

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#### **1. INTRODUCTION**

Soil health or soil fertility is basically a living and dynamic system having all plant nutrients in available forms with an appropriate balance that promotes plant health. Healthy or fertile soil is an ecological characteristic which maintains the quality of air, water and soil environments. Soil fertility shows the form of plant nutrients in the soil whereas the soil productivity is the resultant of various factors in management practices influences crop production (Doran et al. 2000). Soil fertility may be lost through natural process leaching of water-soluble nitrogenous viz.. nutrients, scarce moisture content, wind and water mediated soil erosion, or through human activities viz., exhaustion of nutrients due to monocropping, increase in salinity due to insufficient availability of water, change in soil pH due to inappropriate use of pesticides and fertilizers.

Soils are subjected to pollution due to human activities. Soil pollution or degradation leads to deterioration of soil physico-chemical properties impacting the terrestrial life and thereby lowering food production. Established soil quality or health assessment considers soil physical, chemical and biological parameters or indicators. Soil organisms are an integral part of overall soil productivity and health. Therefore, biological indicators are essential for monitoring soil health (Anderson 2003). Among the useful biological indicators, soil nematodes are an admirable group to study the soil health (Bonger and Ferris 1999. Neher 2001). Entomopathogenic nematodes (EPNs) are among the soil dwelling nematodes which are widely used as biological control agent against insect pests. Various biological characteristics of these nematodes viz., cuticular structure and genetic structure make them useful candidates as bioindicator. The cuticle is permeable and have the restorative capacity of soil ecosystems. Another important feature is the presence of heat shock proteins that are highly conserved in EPNs that can be utilized in determining the level of toxic elements in soil (Hashmi et al. 1997). Expression of these proteins is improved when exposed to stresses such as heat, metal ions, or organic toxins. Nowa-days these proteins are able to serve as biomarkers for eco-toxicological assessment of soils (Kammenga et al. 2000).

#### 2. ENTOMOPATHOGENIC NEMATODES (EPNs)

Entomopathogenic nematodes (EPNs) are important biological control agents against

various economically important insect pests. They are naturally present in different soil habitats. Natural populations of these EPNs keep insect pests population at a certain level and provide food for other groups of invertebrates. Therefore, they are the beneficial soil fauna that take part in the food chain. The genera Steinernema and Heterorhabditis, the obligate parasite of various insect pests are in the families Steinernematidae and Heterorhabditidae respectively under the order Rhabditida. Various species and strains show variation in host range and infectivity as well as survival strategies. "Their occurrence depends on their nutritional preferences i.e., the presence of specific host insect species. The third juvenile stage is the infective juvenile that is free-living in the soil, non-feeding, with closed mouth and anus and capable of surviving for several weeks in the soil, before infecting a new host individual. They have the ability to actively locate their host by recognizing different signals that reveal their presence (vibrations, significant increase in the concentration of CO<sub>2</sub>, volatile specific produced by plants damaged by herbivore, etc.)" (Griffin 2015). "They enter through the natural orifices (mouth, anus, spiracles) of the host and actively penetrate through the midgut wall or tracheae into hemocoel containing haemolymph. They reach the hemocoel and release the symbiotic bacteria (Gram-negative Gamma-Proteobacteria in the family Enterobacteriaceae) that they carry inside, Xenorhabdus in the case of Steinernema and Photorhabdus for the Heterorhabditis species" (Stock 2015). "Nematode and bacteria overcome the insect immune system and the host insect is killed within 48 hours post infection. The bacteria break down the host tissues, and provide food sources for the nematode, which feeds and multiplies on bacterial cells and degrading host tissues. During the process, the bacteria themselves provide a protected role by antibiotics that suppress producina the competition from other microorganisms. The J<sub>4</sub> stage develop into egg laying female or male adults in the insect cadaver and hereby run through four juvenile stages  $(J_1 - J_4)$  and the adult stage" (Stock 2015). "After reproduction and depletion of all nutrients, a high nematode population density triggers the nematode development into IJs again. The lifecycle is completed in a few days and thousands of new IJs emerge, searching for new hosts. Generally, life-cycle of EPNs (infective juvenile penetration to infective juvenile emergence) is completed within 12-15 days. The optimum temperature for growth and reproduction of nematodes is

between 25° C and 30° C. However, the cycle is dependent on temperature and varies for different species and strains. Though they are naturally present in soil ecosystem, their populations can be boosted through controlled releases in areas with pest infestations" (Matuska 2024).

# 3. EPNs AS INDICATOR TO EVALUATE SOIL HEALTH

The soil environment is influenced by mainly human activities. Through land management practices, chemical or physical changes occur to the soil. Recent studies are given that proof that the natural presence of EPN can be an indicator of the impact of soil management (Campos et al. 2008, Blanco et al. 2020).

#### 3.1 EPNs in Different Agricultural Practices

Investigating EPN presence and activity and their relation with other soil organisms associated with them can help us to know the impact of different agricultural practices on crop management system. The soil is a complex, species-rich biological environment and thus, various organisms have the potential to manipulate the survival and reproduction of EPNs. Some of the EPN species are more sensitive to contaminants or disturbances, and their presence or absence can indicate the impact of disturbances on soil health (Lankin et al. 2020). Thus, changes in EPN population structure can imitate changes in soil conditions, such as pollution, or compaction. The higher soil organic matter contents in the field favor the presence of hosts for S. feltiae to allow their long-term persistence. Therefore, reduced tillage or organic farming practices can lead to increased EPN abundance that are indicative of healthier soils (Lankin et al. 2020). "It has been observed that unhealthy soils are related with the excessive use of chemical fertilizers and pesticides, especially in long-term mono-cropping systems. Commonly used fertilizers and chemical pesticides reduce the infectivity of EPNs and negatively affect their reproduction, which reduces the population size" (Matuska et al. 2024). "In an environment in which herbicides were present, EPN reproduction was limited" (Matuska et al. 2024). "Excessive use of inorganic fertilizers contributes to increase soil salinity and accumulation of heavy metals. In saline soil, EPN movement is hindered and their ability to find and identify hosts is limited" (Nielsen et al. 2011). "Long-term

exposure to high concentrations of inorganic fertilizers inhibited the infectivity and reproduction of EPNs. In a study it was observed that NPK fertilizer decreased *S. feltiae* density" (Bednarek et al. 1997).

The survival of IJs is affected by biotic as well as abiotic factors (Lewis et al. 2015). As the soil is a complex ecosystem, introduced EPNs augmented for biocontrol action, may alter the naturally occurring microbiota in the soil (Lewis et al. 2015, Duncan et al. 2007, Ishibashi and Kondo 1986). "The availability of hosts and the presence of competitive organisms and their ecological interaction in the soil are also factors for the prevalence of EPNs in agro-ecosystems" (Stuart et al. 2015). "The occurrence of EPN populations depends on many environmental parameters such as temperature, moisture, soil texture and structure, and pH" (Matuska et al. 2024). "Extreme temperatures result in a decrease in the survival rate of EPNs" (Lewis et al. 2015). "Both high humidity and acidic soil pH reduce populations and infectivity of EPNs. Heavy moist soils are favorable to anaerobic conditions, which, in most cases reduces survival rate and movement within the soil pore. The most favorable soil moisture level is around 25-40% for invasion of the host. Soil moisture also affects nematodes living inside a host insect's body. In dry soil, a small number of juveniles were observed emerging the host body. At low moisture, S. glaseri and S. carpocapsae IJs leave the body of the host insect the earliest, whereas S. riobrave IJs leave the insect body the latest. The structure and texture of the soil also affects the biological function of EPNs. Different species of EPNs differ in their necessities in terms of air-water relations in the soil environment. Soil structures influence the survival, movement and infectivity of nematodes" (Lewis et al. 2015). "Depending on the size of the soil portion, the rate of movement and the frequency of undulating movements changes. In heavier soil, IJ movement is slowed down, whereas in light loam soil they are facilitated. In sandy soil with a mean grain diameter of 0.2-0.5µm, IJs covered a distance of 2 cm (87%) within 48 h; 0.5% of the IJs covered a distance of 12-14cm from the site of release" (Moyle and Kaya 1981). "EPNs especially those are crusier, burrowing and movement activities create channels and pores in the soil, improving the soil structure. Thus, in degraded soils, EPNs can help alleviate compaction and improve water infiltration, aeration and root penetration. Similarly, vertical migration showed that it decreases in response to the increase in clav and silt content, and increases with sandy" (Georgis and Poinar 1983). "S. carpocapsae prefer to migrate downward from the site of application of the IJs, they move deep into the soil, where they effectively infest host insects, whereas H. bacteriophora infests insects above the EPN application site" (Sandner 1986). "It is also observed that IJs penetrate hosts more quickly in sandy and sandy loam soils" (Lankin et al. 2020). "The highest mortality levels of insects were obtained in clay-sandy soils with low moisture and in sand with high relative moisture. Low oxygen concentration decreases infectivity and survival of IJs. S. carpocapsae and S. glaseri IJs placed on sandy, sandy loam, or loam soil for 16 weeks showed different degrees of survival. The survival rate of S. carpocapsae and S. glaseri IJs in sandy loam soils decreased in response to a decrease in oxygen content from 20% to 1%. The lowest survival rate was associated with the loam soil because the low porosity and poor aeration of this type of soil whereas highest survival rate was recorded in sandy loam and sandy soil" (Kung et al. 1991). "IJs use elevated CO<sub>2</sub> concentrations as signals with which to locate a susceptible host" (Kaya et al. 1990). "Soil pH also affects many biological and physic-chemical properties in soils. For the growth and reproduction of most plant species and soil organisms the optimum pH range of soil is 5.5 to 7.2. Acidic soil pH level cause decreases in infectivity and survival of IJs. The survival of S. carpocapsae and S. glaseri was reported to be similar at pH 4, 6, 8 for the first 4 weeks. S. feltiaeand S. bacteriophora showed greater mobility, activity, and pathogenicity at pH 6.8 and 8" (Matuska et al. 2024).

### 3.2 EPNs in Soil Pollutants

environment is "The soil influenced by anthropogenic activities. Anthropogenic activities also influence soil organisms. As the nematodes are exposed to anthropogenic agents, such as heavy metals, oil, gasoline, or essential oils, their slow movement in soil reduces their chances of effectively finding a host. Oil derivatives primarily hydrocarbons, aromatic cause extensive changes in soil biological environment. Various studies evaluated the effect of soil pollution with heavy metals on EPNs" (Ropek and Gondek 2002, Ropek and Nicia 2005). "Petroleum derivatives such as crude oil, diesel oil, engine oil, and gasoline may adversely affect the biology of EPNs All these substances reduce the pathogenicity and reproductive capacity of EPNs"

(Ropek and Gospodarek 2013). "The longer the pollutant is active, the higher the mortality of the nematodes. EPNs could not be isolated from soil contaminated with petroleum derivatives" (Ropek and Gospodarek 2013). "In addition, these compounds inhibit the ability of EPNs to find a host. S. feltiae revealed the greatest sensitivity to soil contamination with petrol at the dose of 8000 mg per kg soil d.m" (Ropek and Gospodarek 2013). "The applied oil derivatives had an adverse effect on the female/male ratio of nematodes infesting host insect" (Ropek and Gospodarek 2013). Assessment can be made with the occurrence of EPNs after application of the biopreparation that reduce a negative effect of soil contamination with oil derivatives.

"Heavy metals are soil pollutants and reduce the suitability of the soil for agriculture. The toxicity of heavy metals and their persistence depend on factors like amount of contamination and the chemical form and other properties of the soil environment like temperature, pH, and presence of other metals. In the soil environment IJs are directly exposed to any heavy metals found there. These compounds affect the occurrence, infectivity, and reproductive capacity of EPNs" (Ropek 2003). "Natural contamination of heavy metals in the soil environment is not the cause of their mortality, but by a decrease in their infection abilities, which adversely affects the success of pest control" (Ropek and Gondek 2002). "Effects of combinations of several metals on S. carpocapsae showed that these combinations reduced the pathogenicity of EPNs" (Ropek and Gondek 2002). "In soils with a higher concentration of heavy metal ions, there is an increased mortality of nematodes inside the body of host insect that causes lower infectivity. The mortality of IJs of S. carpocapsae was affected both by the time of contact of nematodes with pollutants viz., nickel (Ni(II)), lead (Pb(II)), and Cd ion and their concentration. Chloride salts have been found to be particularly toxic" (Jaworska et al. 1999, Jarmul and Kamionek 2003). "Studies showed that the presence of these compounds in soil prevented nematodes from moving, host finding and infecting. Instead of being toxic to nematodes, magnesium ion can act synergistically, by protecting them against the unfavourable impact of other heavy metals" (Jaworska 1999, Jarmul 2003, Jaworska 2009, Jaworska 2014). "Insects exposed to Pb, Cd, or Cu ions, accumulate in the tissues of the insect. also had an unfavorable effect on nematode development in the host, and the viability of IJs emerging from the host. Heavy metals affect not only EPNs, but also the symbiotic bacteria present on them. It was shown that copper, manganese, and nickel ions were most harmful to these microorganisms. In contrast, lead was not toxic to nematode-associated bacteria" (Jaworska 2012, Pezowicz 2002).

#### 4. CONCLUSION AND FUTURE PERSPECTIVES

EPNs present in soil food web, acts as an indirect indicator of the resilience of the agricultural fields or further implementation of good agricultural practices in farming (Blanco et al. 2020). By reducing pest population, EPNs contribute to the overall health of plants. Therefore, monitoring EPNs population during soil health reinstallation efforts can designate the success of restoration activities. EPNs can be integrated with other field management practices and other biological control agents, and insect resistant plant varieties, to create a complete and sustainable pest management strategy that will ultimately improve soil health and food productivity.

#### **DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Author hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

#### **COMPETING INTERESTS**

Author has declared that no competing interests exist.

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