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Utilization of entomopathogenic nematodes in ecotoxicological studies: An overview

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Abstract
Air, water and soil pollutants in the environment disturb the soil ecosystem and harm biological properties of soil. Entomopathogenic nematodes (EPNs) are potential biocontrol agents against many insect pests and are present in soil as infective juvenile stage. This review gives emphasis on the role of EPNs as a bioindicator for ecotoxicological studies in soil ecosystem. Some of the ecotoxicological tools for nematodes like molecular methods and experimental model ecosystem approaches are being adopted for these studies by utilization of EPNs.

Keywords: Entomopathogenic nematodes (EPNs), bioindicator, ecotoxicological studies, environmental pollution, agrochemicals

Introduction

Environmental pollution often occurs in agriculture as well as in industrial sector by diverse pollutants. Many of these environmental contaminants like hydrocarbons, heavy metals, polythenes and various agrochemicals cause health problem of humans and the environment (Brevik *et al*., 2020) [5]. Petroleum-derived substances or oil derivatives present in agricultural soil disturbs the biological properties of soils as well as biotic community and ultimately affects the soil quality and fertility (Kaya, 1990) [26]. Heavy metals, soil salinity, greenhouse effect, eutrophication in water bodies and nitrate accumulation have been found in the areas where agricultural activities are conducted than in natural areas (Susurluk, 2008; Campos-Herrera *et al*., 2010) [36, 8]. Oil derivatives are mainly polycyclic aromatic hydrocarbons which break through the bodies of living organisms and accumulate inside. The abundance of heavy metals in soils also prevents absorption of nutrients and metabolic and physiological processes plant growth, thus reduces the quality and quantity of produce. In the environment, the toxic forms are present for a much longer time period (Kacprzak and Fijałkowski, 2020) [24].

There are different Eco toxicological methods to investigate the mechanisms of toxicity and accurate toxicity threshold level on ecosystem. Both *in vitro* and *in vivo* toxicity tests are done to assess the quality of soil environments. An important reliable tool is the use of living organisms. Various bioindicators such as Nematoda, Collembola, Arachnida or Insecta are used to determine the degree of pollution (Dahiya *et al*., 2022) [12]. Among those, nematodes are of great importance as environmental indicators (Blakely *et al*., 2002) [4]. Soil nematode community is sensitive to pollutants, for this, the interest in nematodes as bioindicator in ecotoxicology studies increased over the past few decades (Georgieva *et al*., 2002; Hagerbaumer *et al*., 2015) [13, 16].

Entomopathogenic Nematodes

Entomopathogenic nematodes (EPNs) of the genera *Steinernema* and *Heterorhabditis* provide an ideal model system for studying environmental impact because of their manipulability, short generation times, and ease of rearing.

Steinernema (*Steinernema*tidae) and *Heterorhabditis* (Heterorhabditidae) under order Rhabditida, are obligate parasites of insect pests and are characterized by symbiotic bacterial species, *Xenorhabdus* spp. and *Photorhabdus* spp., respectively (Adeolu *et al*., 2016) [1]. Infective juveniles (IJs), the only free-living stage in soil, enter the host through natural openings and cuticle (Bedding and Molyneux, 1982; Poinar, 1990) [2, 30]. After penetration into the host's hemocoel, nematodes release their bacteria which are primarily responsible for killing the host within 24-48 h, defending against secondary invaders, and providing nematodes with nutrition. Nematodes complete 2-3 generations within the host, after which IJs exit the cadaver to find new hosts (Kaya and Gaugler, 1993) [28]. EPN pathogenicity to hosts, depends on suppression of the immune system of insects as well as toxin production by symbiotic bacteria (Shaurub *et al*., 2020) [40].

Impact of Ecotoxicological factors on EPNS

Different ecotoxicologial factor affect the survival, development, and reproduction of EPNs, and thereby reducing infectivity. Environmental variables, agricultural management or land use systems, soil characteristics are known to affect their distribution and occurrence (Selvan *et al*., 1993) [38]. A low abundance of EPNs are found in agricultural fields as compared to natural fields. The use of chemical pesticides, chemical fertilizers, and or fresh manure has been shown to have detrimental effects on the. Shapiro *et al*., 1997 [39]; Şahin and Susurluk, 2018 [36] observed that survival and bioefficacy of EPNs reduces due to excessive and prolonged use of fertilizers. There are differences in survival of EPN species or strains under exposure to various pollutants. Bednarek and Gaugler (1997) [3] observed that *Steinernema Feltiae* was more resistant than H. *bacteriophora* (HBH) to inorganic fertilizers. Soils with increased levels of some of the elements may lead to reduced occurrence of EPNs. Kawaka *et al*., (2014) [25] observed field soil samples from Taita with levels of C, N, Mg, P, Mn and Fe had a negative effect on the EPN occurrence. A soil survey in La Rioja (Northern Spain) found that natural entomopathogenic population densities were negatively correlated with Zn and Cu concentrations (Campos-Herrera *et al*., 2010) [8]. Campos-Herrera *et al*., (2008) [9] observed that *S. carpocapsae* was sensitive to Cu and Zn but H. *bacteriophora* was not affected. Laboratory studies documented a direct lethal effect of certain metal ions on EPN activity (Jaworska *et al*., 1996; Salamun *et al*., 2012) [22, 37] and reproductive capacity and infectivity (Jaworska *et al*., 1999) [21]. The amounts of Pb, Cu, Cr, Fe, Mo, Ni, Va, Cd, Zn and Li negatively affect virulence and infectivity of EPNs (Jaworska and Gorczyca, 2002; Sun *et al*., 2016) [19, 41]. Jaworska *et al*., (1997) [23] in a laboratory study observed positive correlation between Magnesium and H. *bacteriophora*. However, Mn slightly stimulated the reproduction of S. *feltiae* (Jaworska *et al*., 2002) [19]. Jarmul and Kamionek (2000) [17] observed high mortality of S. *feltiae* and H. *megidis* and decreased infectivity in relation to the test insects on the exposure to lead ions. More input of fertilizer can negatively effect on EPN occurrence, and continuous exposure to these pollutants might lead to natural EPN population extinction. Though Sodium (Na) did not have any impact on the nematode population but has been reported to kill developing nematodes (Kaya and Stock, 1997) [27]. Similarly, different salts like NaCl, KCI and $CaCl_2$, have different affect on S. *glaseri* and H. *bacteriophora* differently. But in S. *glaseri* its efficiency, virulence and penetration remain unaffected by these salts. Effects of NaCl on H. *bacteriophora* impart the toxicity and possibly interfere with the host finding behavior (Thurston *et al*., 1994) [43]. Iron (Fe2+) have been reported to have low effect on the infectivity of the nematodes with respect to *Galleria mellonella* (Jaworska *et al*., 1997) [23]. Patel and Wright, (1996) [29] reported the occurrence of EPN natural populations in natural areas and crop fields when exposed to organochlorine pesticides. Shapiro *et al*., (1997) [39] and Campos-Herrera *et al*., (2008) [9] showed that p, p0-DDE, g-BHC and hexachlorobenzene (HCB), clay, P_2O_5, Zn, and Cu contents negatively correlated with EPN population density. Chelinho *et al*., (2011) [11] and Touray *et al*., (2021) [44] observed the higher sensitivities of Diplogasteridae and Rhabditidae in carbofuran-treated microcosms study. Entomopathogenic nematodes are also susceptible to oil derivatives, which affect mortality and infectivity (Ropek and Gondek, 2002) [32], making them a good candidates as indicators for this kind of pollution as for example soil exposed to oil derivatives had a negative effect on S. *feltiae* to penetrate *Tenebrio molitor* larvae.

Ecotoxicological Tools for EPNS

Now-a-days molecular tools are being used to identify and quantify EPNs and other members of the soil food web (Campos-Herrera *et al*., 2013) [6]. Therefore, these molecular tools can be used to test the toxicity of the accumulated toxic elements on EPN biology on a species specific basis. Soil moisture is an important abiotic factor affecting EPNs populations, and management of soil moisture has the greatest potential to enhance and conserve EPNs (Campos-Herrera *et al*., 2022) [7]. The interaction of soil moisture and accumulation of toxic elements on EPNs and other soil biota needs to be studied.

Bioremediation is an important process, using microorganisms or living organisms or microorganisms in combination with vegetation. Bioremediation is an efficient, widespread, cost-effective, and eco-friendly tool to decontaminate the environment (Ropek and Gospodarek, 2022) [35]. Bacteria and nematodes are used for the biodegradation of oil derivatives polluting water environments. Monitoring should be done during the remediation process. Various hydrocarbon-degrading microorganisms are used which involves specific microbial enzyme systems like oxygenases, peroxidases, dehydrogenases, and hydroxylases which are considered enzymatic key reaction catalysts in biodegradation (Chan, 2011) [10]. Gospodarek *et al*., (2016; 2021) [15, 14] suggested the application of biopreparation ZB-01 as a bioremediation process. Ropek and Gospodarek (2013) [34] found that the use of a microbial preparation had a beneficial effect on the natural occurrence of entomopathogenic nematodes in contaminated soils, as they were isolated earlier than in the sections undergoing the process of natural remediation. The mortality of test insects caused by the nematode S. *feltiae* was a good indicator of the progress of bioremediation of petroleum pollutants, such as diesel oil and engine oil. The application of biopreparation accelerated the bioremediation process and diminished a negative effect of soil pollution with engine oil on virulence of *S. feltiae* nematode. Petrol usually pollutes the soil for a shorter time than other oil derivatives. Research demonstrated that a negative effect of soil contamination with petrol on the ability of S. *feltiae* to kill test insects and penetrate their bodies persisted for a relatively short period of time after initial contamination.

Conclusion

The treatment of pollutants from contaminated soil or water by bioremediation is an achievable process. EPNs occurrence and population density is one of the parameters in the ecotoxicological studies. In the ecological risk assessment of various bioremediation techniques theses parameters are of great importance.

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