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Entomopathogenic Nematodes: A review

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Abstract: The aim of study is data collection about entomopathogenic nematodes and mutual bacteria and their effect on insects caused harmful to plants and the mechanism they cause in killing insects.

Keyword: Entomopathogenic Nematodes , Heterorhabditis ,Steinernema , *Xenorhabdus* sp. , *Photorhabdus* sp.

Introduction:

Agricultural pests cause significant losses in agricultural crops and food products, whether in fields, orchards, or in warehouses. Perhaps one of the most important and most dangerous agricultural pests is insects, which are responsible for causing about 28% of the damage to agricultural crops in fields, orchards and stores (Abu Tara, 2008) Pest control is one of the aspects of agricultural development, and the pioneer in that was the use of chemical pesticides, and companies began to compete to produce various types of pesticides in huge quantities , Farmers continued to spray dense and indiscriminately in quantity and quality and in different ways until it was mixed with irrigation water, and the indiscriminate use of pesticides led to the emergence of many negative effects, including pollution of the environment, the emergence of many dangerous diseases that affect humans, killing many natural vital enemies that led to the disturbance of balance Natural among pests and their natural enemies, as resistant strains of insects appeared to chemical pesticides, and many insects have turned into primary pests that cause many damage to crops, so it was necessary to return to the ecosystem and deal with it rationally and not resort to introducing foreign and new materials as much as possible to maintain cleanliness. As a result, the concept of Integrated Pest Management (IPM) emerged, which adopts Biological control as its main focus through the use of living organisms (predatory insects, intrusive insects and insect pathogenic microorganisms) to prevent or mitigate Losses or damages resulting from pests by affecting the density of the pest and the reduction of disease Its stems to the harmless level (Ehlers, 2015).

Insect-pathogenic nematodes (EPNs) were first discovered in the 1920's and received increasing attention starting in the 1950's and marketed in the 1980's, due to their high safety for humans and their lack of impact on non-target organisms and the environment. They differ from pesticides in terms of negative environmental impacts (Ehlers). , 2005; Piedra Buena et al., 2015). It is for this reason that we note the commercialization of EPNs, on at least 5 types of Heterorhabditis and 8 types of Steinernema (a lot of research on the application, biology and ecology of EPNs has been done extensively since the 1990s and continues to this day. It spread to the United States in the year 2000).(Gaugler and Kaya, 1990; Bedding et al., 1993; Gaugler, 2002; Grewal et al., 2005); Campos-Herrera, 2015) was used as our primary references. .

Increasing a lot From the end of the 1960s and the beginning of the eighties of the twentieth century, focused and intensive research began that clarified the importance of Entomopathogenic nematodes and their role as highly efficient biological control agents (Bedding et al, 1993; Gauglar, 2002; Kaya and Koppenhofer, 2004). After that, much interest in the group of insect pathogen nematodes increased.

The study aimed to collect information about the most important insect pathogen species and to identify them for a later use as an alternative to insecticides that have a harmful effect on the environment and on some other non-target neighborhoods, and to prevent the emergence of insect strains resistant to those pesticides.

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Classification of Entomopathogenic Nematode:

There are approximately 30 families of nematodes that are associated in one way or another with insects (Adams and Nguyen, 2003; Nguyen and Smart, 2004). Some of these families include genera and species that have proven to be highly effective as insect pathogens and some have latent capabilities to an acceptable degree (Bedding et al, 1993; Gauglar, 2002 ; Kaya and Koppenhofer, 2004). Below is a table showing some Order, families, and Genus of insect pathogenic / parasitic nematodes**.**

Table (1) Some Order, families and Genus of the nematode pathogenic / parasitic on insects.

Order	family	Genus
Rhabditida	Steinernematidae	Steinernema
		Neosteinernema
	Heterorhabdidae	Heterorhabditis
	Rhabditidae	Rhabditis
	Diploscapteridae	Diploscapter
Diplogasterida	Diplogasteridae	Mikoletzkya
Tylenchida	Allontonematidae	Allantonema
	Sphaerulaiidae	Sphaerularia
	Parasitylenchidae	Parasitylenchus
	Phaenopsitylenchidae	Deladenus
	Iotonchidae	Paraiotonchium
	Fergusobiidae	Fergusobia
	Entaphelenchidae	Entaphelenchus
Oxyurida	Thelostomatidae	Blatticola
	Travassosinematidae	Travassosinema
	Hystriganthidae	Hystrignathus
Mermithida	Mermithidae	Romanomermis

Note: Not all orders in this table have been demonstrated to be parasites or insect pathogens.

Table (1) includes 17 families follow six order, and the first five follow the Class: Secernentea, while Order: Mermithida follows the Class: Adenophorea (Nguyen and Smart, 2004; Adams and Nguyen 2003), Not all families in this table have been shown to be pathogenic as parasites or insect pathogens, but some research and reports have considered them as insect parasites.

The following families (Steinernematidae, Heterorhabdidae, Phaenopsitylenchidae) are characterized by their high efficiency and are used in biological control of insects, and their advantages are that they live symbiotically with microorganisms that increase their disease efficiency, the possibility of intensive multiplication on a commercial scale, their good ability to survive and adapt in the field. (Al-Hazmi ,2008)

As for the following families (Mermithidae, Iotonichidae), they showed a degree of potential in biological control and a few of their species are used in insect control. While the following families (Allantonematidae, Diplogasteridae, Parasitylenchidae, Sphaerulariidae, Tetradonematidae, Rhabditidae) are likely to be successful as biological control agents for pests and are characterized by their difficulty in propagation and poor survival in the field as well as the presence of other characteristics that limit their use in biological control. There is another group of hosts (Carabonematidae, Syrphonematidae, Oxyuridae, The lastonematidae, Aphelenchoididae, Entaphelenchidae, Fergusobiidae) that live with insects and have low pathogenicity and do not have the inherent ability to control insects.(Al-Hazmi ,2008).

Nematodes and bacteria and their relationship(*Xenorhabdus* for Steinernematidae and *Photorhabdus* for Heterorhabditidae)

In this paper, we focused on entomopathogenic nematodes in the families Steinernematidae and Heterorhabditidae. The family Steinernematidae includes the following genera Steinernema and Neosteinernema while the genus Heterorhabditis belongs to the family Heterorhabditidae (Kaya and Gaugler, 1993; Kaya and Stock, 1997; Burnell and Stock, 2000). These genera are related to bacteria, for example

bacteria genus Xenorhabdus related to the family Steinernematidae and Photorhabdus related to the family Heterorhabditidae (Boemare, 2002), the presence of nematodes with bacteria in the insect host act together as a biological controller for insect killing.
Bacteria are present in specific areas of the nematode body. Bacterial cells are observed in a vesicle in the anterior part of the intestine for Steinernematids and in the intestinal tract for Heterorhabditids (Hazir et al; 2003).

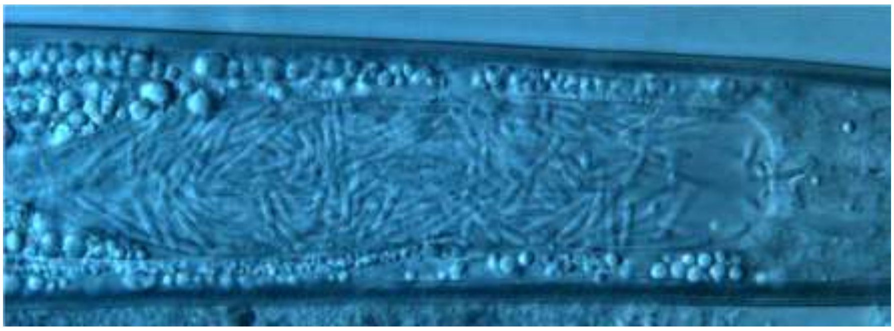

Figure 1. *Xenorhabdus hominickii* in *Steinernema monticolum*
(http://contents.kocw.or.kr/KOCW/document/2014/kangwon/kimsamgyu/11.pdf)

Nematodes enter the host through natural openings or thin areas of the host's epidermis (mouth, respiratory openings, anus) and this is observed in the genus Heterorhabditids (Kaya and Gaugler, 1993) as they penetrate the hemocoel of the insect. The infected nematode then releases the bacteria through the anus of Steinernematids (Poinar, 1966) or through the mouth of Heterorhabditids (Ciche and Ensign, 2003). Soon, bacteria that live with nematodes multiply mutually to produce substances that kill the insect quickly, in addition to these substances protect the body from attacking other microorganisms. Pathogenic nematodes begin to develop, feeding on bacterial cells and host tissues Nutrients are consumed in the host insect, a new generation of infective larvae is produced and emerges from the infected insect’s body into the soil in search of a new host (Figure 2).

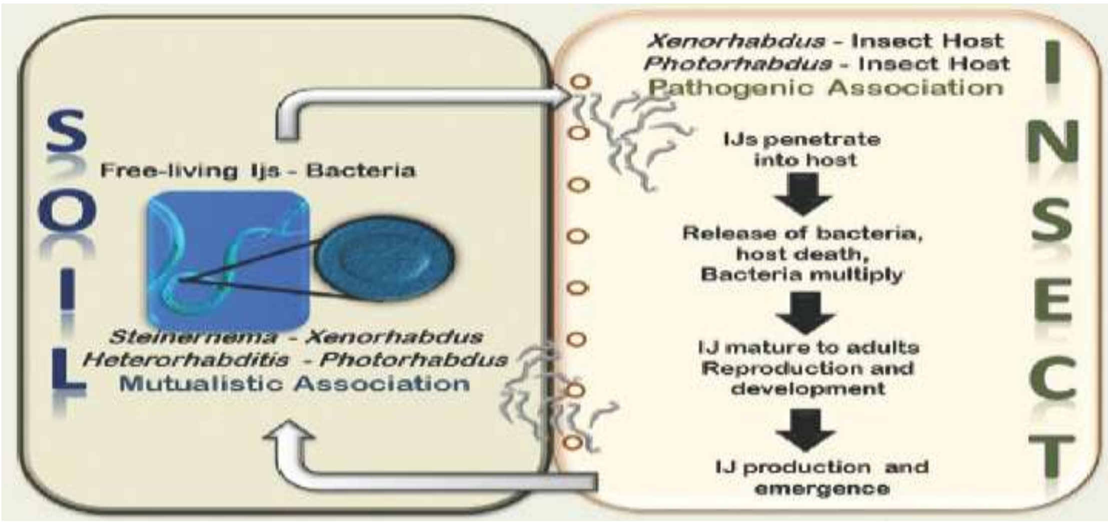

Figure 2. Life cycle of entomopathogenic nematodes (Orozco et al; 2014)

The relationship between nematodes and bacteria is indeed bidirectional for the following reasons: nematodes rely on bacteria to create the right to an environment for its development through the formation of antibiotics that inhibit the growth of competing microbes and host tissues, and quickly kill insects. Bacteria need nematodes to protect themselves from the external environment, penetrate the host's blood cavity, and inhibit the host's antimicrobial proteins (Hazir et al; 2003)

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Mutually beneficial bacteria:

Xenorhabdus and *Photorhabdus* are anaerobic, -ve gram, non-sporulated bacillus anaerobic bacteria, facultative, in the family Enterobacteriaceae. In the genus *Xenorhabdus*, there are five species related to *Steinernema*, while in the genus *Photorhabdus*, three species are related to Xenorhabdus elegans (described by P. luminescens, P. tempata, and undescribed P.) (Burnell and Stock, 2000; Boemare, 2002; Fischer- Le et al; 1999; Hazir et al; 2004) with one species, *P. luminescens*, divided into 5 subspecies. The subspecies of *P. luminescens* is a subspecies. *luminescens*, *laumondii*, *akhurstii*, *kayaii* and *thraciaensis*. (Boemare, 2002).

There are significant differences between the two bacterial genera (Boemare, 2002). Of which most are *Photorhabdus* spp. It is catalase-positive and luminescent while in *Xenorhabdus* spp. it is catalase-negative and luminescent. Each species of bacterial genera has different phenotypic cells called primary form (stage I) and secondary form (stage II) (Forst and Clarke, 2002). The primary form is the cell type naturally associated with the nematode, while the secondary form can arise when the bacteria are in a stationary non-growth stage. The secondary form of *Xenorhabdus* to the primary form, this phenomenon was observed in *Photorhabdus* spp. no logs. Monooxygenase is a linker between bacteria and nematodes but other bacterial species have been isolated from infected larvae of various stone nematodes (Lysenko and Pfizer, 1974; Aguillera and Hodge, 1993; Aguillera and Smart, 1993) and *Xenorhabdus elegans* (Jackson et al.; 1995; Babek et al.; 2000) species. In recent studies of *X. nematophila* and *S. carpocapsae* by Vivas and Goodrich-Blair (Vivas and Goodrich-Blair, 2001) it was observed that bacterial genes are used to maintain specificity between bacteria and nematodes, Martens et al. (2003) A small number of *X. nematophila* cells begin to colonize an infective juvenile and thus have been observed to be increasingly present within the intestinal lumen and develop in a reproducible multiphase pattern during colonization.

The effect of Mechanism Entomopathogenic nematodes:

The families Steinernematidae and Heterorhabditidae are considered one of the most important families of entomopathogenic nematodes (EPNs), and they are safe on vertebrates and plants without negative effects on the environment and are considered one of the effective factors in biological control against a number of important insects in other soils (Al-Hazmi, 2008)

The third stage enters the insect's body cavity through its natural openings, the nematode secretes quantities of bacteria - which live with nematodes symbiotic living - inside the insect's bloody fluid, and these bacteria cause blood poisoning that leads to the death of the insect. (Al-Hazmi ,2008)

The nematode feeds on bacteria that multiply in the insect's blood, in addition to feeding on the insect's tissues and multiplying inside the infected insects in preparation for their growth and development, and the exit of the third larval stage in huge numbers from the dead insect's body to infect another insect. (Al-Hazmi ,2008)

Symptoms of insects infected with nematodes and bacteria:

The dead insects appear with nematodes for a long time intact and do not have a musty smell, but they are colored yellowish-brown or red, and the body tissues appear resinous. Infected insect, *Xenorhabdus* bacteria are found in members of the family Steinernematidae and *Photorhabdus* bacteria are in members of the family Heterorhabditidae.(Sushil et al; 2020)

The relationship between bacteria and nematodes is a symbiotic relationship or mutual benefit, wherein the entomopathogenic bacteria benefit from the nematode in that they carry them inside the body of the insect, while the bacteria prepare the body of the insect to suit the growth, reproduction and feeding of the nematode, knowing that these bacteria are not harmful to vertebrates and are found only in nematodes and insects infected with them. It was not found free in the soil at all. .(Sushil et al; 2020).

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Figure 3. Symptoms of insect . (a) Uninfected larvae of the mealworm *Tenebrio molitor*; Below: worm infected with nematodes *Steinernema feltiae* (Eilenberg et al; 2015) , (B) uninfected larvae (left) ; (right) : infected with *Heterohabaditis bacteriophora* (http://contents.kocw.or.kr/KOCW/document/2014/kangwon/kimsamgyu/11.pdf)

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