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Identification and assembly of entomophagous nematodes in the cold temperate forest of the high mountain range of Zacualtipán de Ángeles Hidalgo

Identitificación y montaje de nematodos entomófagos bosque templado frío de la sierra alta de Zacualtipán de Ángeles Hidalgo

VILLEGAS-VELAZCO, Moises*†, ORDOÑEZ-LOZANO, Itzcoatl and SAN JUAN-LARA, Jorge

Universidad Tecnológica de la Sierra Hidalguense. Mexico-Tampico Highway, Km. 100 Colonia. Pachuca-Huejutla section. Zacualtipán de Ángeles, Hidalgo, México, C.P. 43200. Mexico.

ID 1st Author: *Moises, Villegas-Velazco*

ID 1st Co-author: *Itzocoatl, Ordoñez-Lozano*

ID 2nd Co-author: *Jorge, San Juan-Lara*

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Abstract

The study on the identification of nematode entomophages was carried out in the forest of Zacualtipán of Angeles, Hidalgo, property of the Technological University of the Sierra Hidalguense. The problematics presented in the zone is the application of chemical pesticides that damage to the environment, since they kill many charitable organisms and even, they concern the health of the human being. To solve this problem, it was done a research on the morphological identification by means of microscope of nematode entomóphages that are present in the enclosed floor to the good healthy pine roots. This was achieved using the techniques of having sifted, flotation and permanent fixation, it was also made a count and a calculation of the percentage of this type of microorganisms. Being a great variety of nematode entomophages that can serve in a future as biological control of plagues that damage to the timber production. The impact looked is that there could be generated biological nice pesticides to the environment from the studies done in this work.

Entomophages nematodes, sifted, flotation, permanent fixation, Zacualtipán

Resumen

El estudio sobre la identificación de nematodos entomófagos se llevó acabo en el bosque de Zacualtipán de Ángeles Hidalgo, propiedad de la Universidad Tecnológica de la Sierra Hidalguense. La problemática que se presenta en la zona es la aplicación de plaguicidas químicos que dañan al medio ambiente, pues matan muchos organismos benéficos e incluso afectan a la salud del ser humano. Para solucionar este problema se realizó una investigación sobre la identificación morfológica mediante microscopio de nematodos entomófagos que se encuentran presentes en el suelo adjunto a las raíces de pino en buen estado de salud. Esto se logró utilizando las técnicas de tamizado, flotación y fijación permanente además se hizo un conteo y un cálculo del porcentaje de este tipo de microorganismos. Encontrándose una gran variedad de nematodos entomófagos que pueden servir en un futuro como control biológico de plagas que dañan a la producción maderera. El impacto que se busca es que se puedan generar plaguicidas biológicos amables al medio ambiente a partir de los estudios realizados en este trabajo.

Nematodos entomófagos, tamizado, flotación, fijación permanente, Zacualtipán

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* Correspondence to Author (Email: moy28_82@hotmail.com)
† Researcher contributing first author.

Introduction

Nematodes are organisms of the animal kingdom that are basically found everywhere. Entomophagous nematodes are organisms that feed on other insects, mites, or other arthropods, which have multiplied massively and are released in the fields against pests of these same groups. They are called natural enemies of pests, and naturally fulfil the function of reducing the populations of phytophagous pests, reducing their possibilities of developing and producing economic damage.

In the Zacualtipán region it has not been possible to carry out an adequate study to indicate if these organisms are present in the forest, as well as to obtain information on the benefits of entomophagous nematodes to the trees. The forest is an especially important source of economic resources, due to the forest exploitation, as well as a valuable ecosystem that possesses soil, water, fauna, and air in good quality. However, the forest area is reduced year after year, due to various causes, such as immoderate logging, forest fires and the attack of pests and diseases.

The species used for forestry in the municipality of Zacualtipán are threatened by pests, so the aim is to study the forest species of Pinus patula found in the cold temperate forest of the central sierra of Zacualtipán, Hgo. The aim of the study is to locate the genera of phytoparasitic nematodes that are present in the roots of *Pinus patula* so that the necessary preventive measures can be taken to prevent diseases from developing in other tree species and causing both economic and environmental losses to the ecosystem.

The project aims to analyse the soil attached to the roots of *Pinus patula* in the forest of the central highlands of Zacualtipán, to search for entomophagous nematodes present to identify and describe their population. This information will be of great help in the prevention and control of pests in the crops surrounding the ecosystem.

By carrying out these activities, we are helping to understand and maintain the forests in good condition, free of diseases and pests, obtaining great economic benefits due to the systems of sustainable forestry, since the forests are in good condition, obtaining a good timber harvesting and good quality, the ecosystem will also benefit, since the ecological balance between the various species will be better understood and thus will seek their conservation.

The aim of the soil study is to analyse the genera of nematodes associated with pine trees to combat pests that damage them.

Theoretical background

Description of nematodes. Nematodes are one of the most diverse species on the planet, with more than 20,000 species classified. They live in all parts of the world. They survive in freshwater, saltwater, and all types of soil. They are present in various types of climates, from tropical forests to polar ice caps, from underwater pits to desert sands, from ocean beaches to mountain peaks. The word nematode comes from the Greek words "nematos" meaning "thread", and "eidos" meaning "thread".

Meaning "form" which is very explicit, all these species share the basic characteristics of being structurally quite simple. They are divided into two main groups: chewers: they eat and devour their prey. Suckers: they absorb and suck the juices of their prey. [3]

Nematodes are thread-like organisms of the animal kingdom, colourless, unsegmented, lacking appendages; they can be free-living, parasitic, or predatory and are generally microscopic. They have digestive organs and glands, reproductive and excretory structures, muscular system, nervous system, cuticle.

The word nematode derives from the Greek word "nema", which means "thread-like", as they have an elongated, cylindrical body. As a result of their evolution, nematodes have adapted to explore a wide variety of food sources. Thus, parasitic nematodes can colonise and feed on plant and animal tissues, while free-living forms feed on micro-organisms (bacteria and fungi), protozoa, algae, other nematodes, and micro-invertebrates.

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They move by zig-zag muscle contractions and extensions, swimming on the ground with a film of water. They are found everywhere and in all types of habitats and with different feeding habits: animal parasites, human parasites, plant parasites, predators, entomophagous, mycophagous, bacteriophagous and free-living [1].

Entomophagous nematodes

Entomophagous nematodes: They belong to the Family: Mermithidae. Entomophagous nematodes are useful for insect control. The Families Heterorhabditidae, Mermithidae and Steinernematidae.

They are Main genera and species used as potential biological control agents [2].

Organisms that feed on other insects, mites, or other arthropods, which have multiplied massively and are released in the fields against pests of these same groups, are called natural enemies of pests, they naturally fulfil the function of reducing phytophagous populations, reducing their chances of developing and producing economic damage.

Entomophagous nematodes are more complex than entomopathogenic micro-organisms, developing through four larval moults before reaching maturity; adults are bisexual.

Entomophagous nematodes attack different groups of insects. They require a certain moisture environment for active infection; they usually act on insects that have part of their life cycle in the soil, where moisture is higher [5].

Three types of entomophagous nematodes can be distinguished and are described below:

1. More primitive nematodes. These are pathogenic but eat bacteria of the Rhabditoides group. The nematode penetrates the insect and secretes into it a bacterium with which it is associated. The nematode feeds on the bacteria it secretes, which develop inside the insect. Nematodes of the genera *Neoaplectana* and *Heterorhabditis* are prominent.
2. Evolved nematodes. These are plant pathogenic nematodes that have evolved into insect pathogenic forms. They use the stylet to penetrate the insect. The main groups are the Aphelenchida and Tylenchida.
3. Predatory nematodes. They belong to the Dorylaimida group. Commercially, *Neoaplectana carpocapse*, which affects Lepidoptera and Coleoptera, and *Heterorhabdilis* spp. in Lepidoptera, are the most important.

The life cycle and reproductive habits of parasitoids are often extraordinarily complex. In some species only one individual grows inside its host. In other cases, hundreds of young larvae develop inside the insect pest.

A parasitoid needs a host to complete its life cycle. The adult matures and the host dies. [4].

Characterisation

The nematodes most used in biological control (Heterorhabditidae and Steinernematidae) have an infective juvenile stage that protects and carries a bacterium of the genus *Xenorhabus* that is released into the haemocoel. The infective juveniles enter the insect via the spiracles, anus or mouth and pass into the haemocoel, via the gut wall, where they inoculate the bacterium which kills the host after 1 to 2 days. Depending on the host, certain species of *Heterorhabditis* can penetrate directly through the cuticle (see Fig. 1, 2). (See Fig. 1, 2) [1].

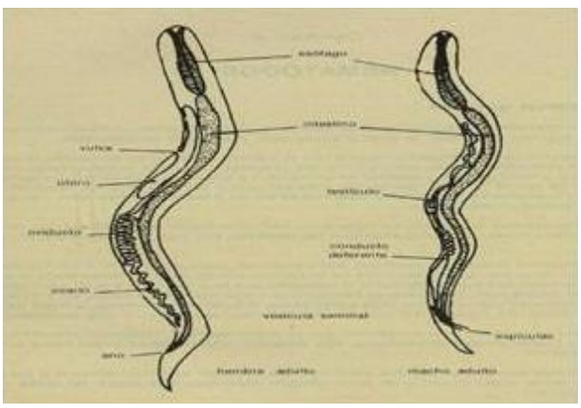

Figure 1 General morphology of nematodes

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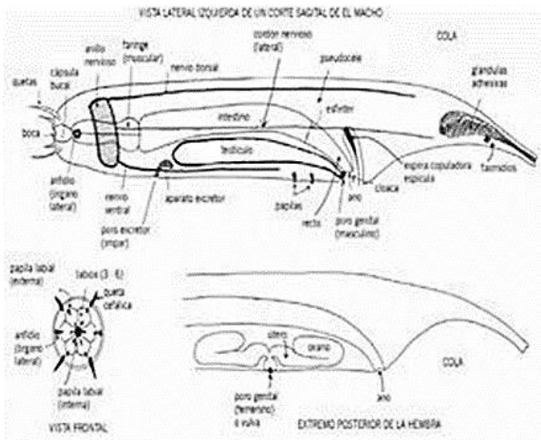

Figure 2 Nematode morphology

Methodology

A systematic search for nematodes was carried out in the soil attached to the roots of various trees in the forest of the Universidad Tecnológica de la Sierra Hidalguense, where nematodes were extracted from the soil by sieving, centrifugation, and flotation in sugar solution. The nematodes were then isolated, killed, fixed, dehydrated and permanently mounted for identification. Identification was made by microscopic morphology.

Soil samples were collected in May 2014. In each plot, 10 samples were taken at a depth of 5 cm from the soil surface, each sample weighed 1 kg, then in the laboratory the samples were sieved to remove clods and organic matter. Each sample was subdivided 7 times, each sub-sample weighed 50 g and was poured into 100 ml plastic cups. After sieving, 5 to 15 mL of sterilised water was added to moisten the soil without reaching saturation point. Finally, they were left to stand for 2 hours at room temperature.

Isolation of microorganisms

The bait insect technique (Zimmermann 1986) was used for the trapping of entomopathogenic microorganisms in soil. Five Tenebrio molitor larvae per beaker were incubated. Samples were examined every day for one month. When the larvae showed symptoms of infection, they were incubated in petri dishes with moist paper to allow their displacement and survival of the nematodes.

Results

Using the bait insect technique for trapping entomopathogenic microorganisms, *Steinernema* nematodes were found. (See Fig. 3 and 4).

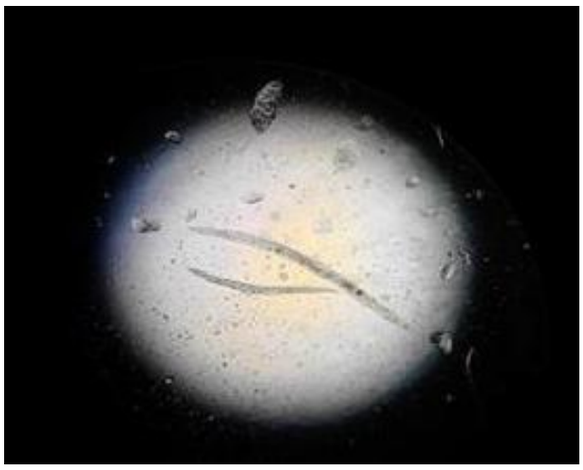

Figure 3 *Steinernema* nematodes

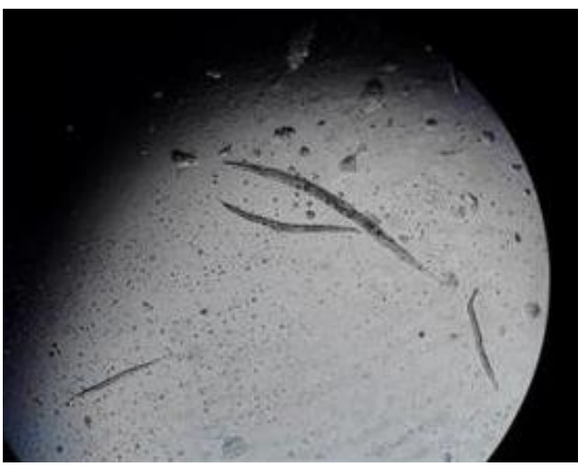

Figura 4 *Steinernema* nematodes

Out of 17 samples taken from one hectare of forest at the Universidad Tecnológica de la Sierra Hidalguense, 241 nematodes were found, of which 71.78% could not be analysed because the necessary equipment was not available, therefore they were classified as Non Entomophagous, of the remaining 15.35% are *Neosteinernema*, 12.45% are *Steinernema* and finally 0.41% are *Heterorhabditis* as shown in the following table:

Nematode type		Quantity	%	Total (%)
Not entomophagous		173	71.78	71.78
Entomophages	*Neosteinernema*	37	15.35	28.22
	Steinernema	30	12.45	
	Heterorhabditis	1	0.41	
Total		241	100.00	100.00

Table 1

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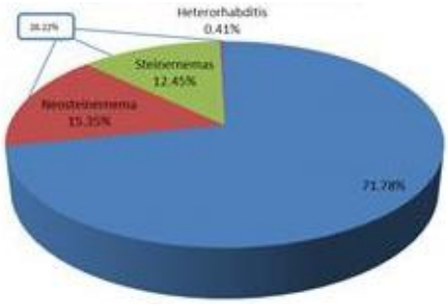
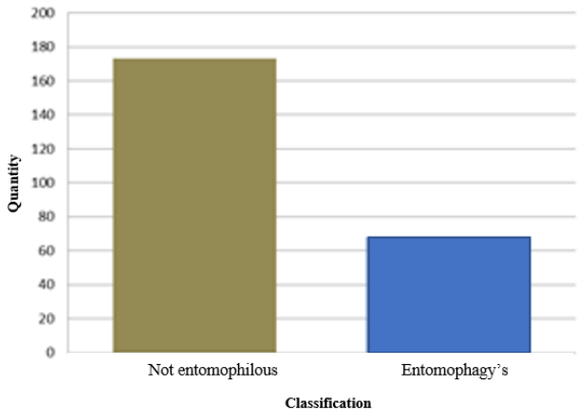
The results obtained can be better appreciated in the following pie chart, which shows the percentage of each type of nematode obtained (see Graphics 1 and 2).



Graphic 1 Classification of nematodes



Graphic 2 Quantification of nematodes

Interpretation

In total 241 nematodes were found, 68 entomophagous nematodes were identified and it could be observed that the presence of predatory nematodes is relatively considerable compared to the other types of nematodes, but it is still particularly important as it controls the other populations. It is important to emphasise that using nematicides will also kill nematode populations that balance the insect population, which is something that should be considered by professionals seeking to control nematode pests.

Conclusions

In the studies carried out around the Universidad Tecnológica de la Sierra Hidalguense, 241 nematodes were identified, of which 68 are entomophagous nematodes, and it was observed that the presence of predatory nematodes is relatively considerable to create ecological pesticides that do not harm the environment or mankind, favouring the development of trees and thus improving the quality of wood obtained from these forests.

All this analysis helps agricultural and forestry producers to have a valid tool for the treatment of infested plants of economic interest. Further research is needed.

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