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Entomopathogenic Nematodes: Biological Model of Studies with Anthelmintics

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Abstract

Anthelmintics used in animals to combat parasitic infections are mainly excreted in manure and cause negative effects on the environment and decomposers. Nematodes are associated with the rhizosphere; some are gastrointestinal parasites of animals, and others regulate insects and other arthropods (entomopathogenic nematodes) and are considered beneficial. The habitat and the similarities that exist among them give the opportunity to use nematodes as a biological model. The availability of target organisms is not always feasible; therefore, experimental studies with models similar to those of the target organisms are a possibility. In veterinary clinics, the study of drug susceptibility is a fundamental tool to monitor the development of resistance. To conserve the biodiversity of the environment, it is necessary to make adequate use of anthelmintics, avoid resistance to these pesticides and prevent the used products from damaging populations of beneficial organisms.

Keywords: Rhizosphere, parasites, intestinal, entomopathogens, anthelmintics, resistance

1. Introduction

Nematodes in nature show astonishing biodiversity since only approximately 30,000 species have been described, but it is estimated that there are a million or more species of nematodes worldwide. Nematodes generally have a long, narrow and thread-like body ('nema' from the Greek 'thread'), not segmented like earthworms. Its body is basically tubular, and the intestine and gonad are surrounded by the wall of the body with its dorsal and ventral longitudinal muscles, epidermis and cuticle. Between the inner and outer tubes, there is a pressurised cavity filled with fluid that acts as a hydrostatic skeleton, all of which allows the nematodes to move in sinusoidal waves. The morphological diversity in this group is restricted and much lower than that of other arthropods or vertebrates. All nematodes go through three or four larval stages, and at the end of each stage, a new cuticle is synthesised, and the previous cuticle is moulted. The nematode species are very diverse, and the most obvious differences are observed in size, which varies from fractions of a millimetre to several metres, cuticular decorations and especially feeding structures.

The mouth of nematodes can be a simple tube, or it can be decorated with a perforating stylet (in plant parasites and fungal feeders) or with teeth that can cut, tear or bite in predatory species such as *Mononchus* and in some intestinal parasites such as *Strongylus* [1].

Nematodes are also the most numerous group of parasites of animals and humans and are widely distributed in a variety of habitats. Some are free-living, while others are in some part of their life cycle parasites of plants and vertebrates or invertebrates [2]. Parasitic nematodes of animals have great economic importance; due to the high frequency with which they occur, they are generally chronic and most interfere with good growth. They can be located in most organs, mainly in the digestive tract, have a direct or indirect life cycle, and some are zoonotic [3].

2. Anatomy

The cuticle is a noncellular, flexible and elastic structure that generally has externally arranged rings; however, since the cuticle is not visible, it has a smooth, shiny appearance and is secreted by the layer of cells that are immediately below, that is, the hypodermis (**Figure 1**). The cuticle is formed by several layers whose number and thickness vary according to the species in question and is composed of proteins such as albumin and glycoproteins [4].

The hypodermis is a thin layer of four tubular thickenings, called the dorsal cord, two lateral and one ventral. It contains cells that secrete the layers of the cuticle. The muscular system is composed of two types of muscles, specialised and nonspecialized or somatic, which occupy a position close to the hypodermis of the areas between the cords, forming a single layer of cells, which has an important role in body movements [5].

The specialised muscles are found in several positions and have important functions, such as the oesophageal muscles in the wall of the oesophagus, the intestinal muscles in the wall of the intestine, the dilator and compressor muscles of the

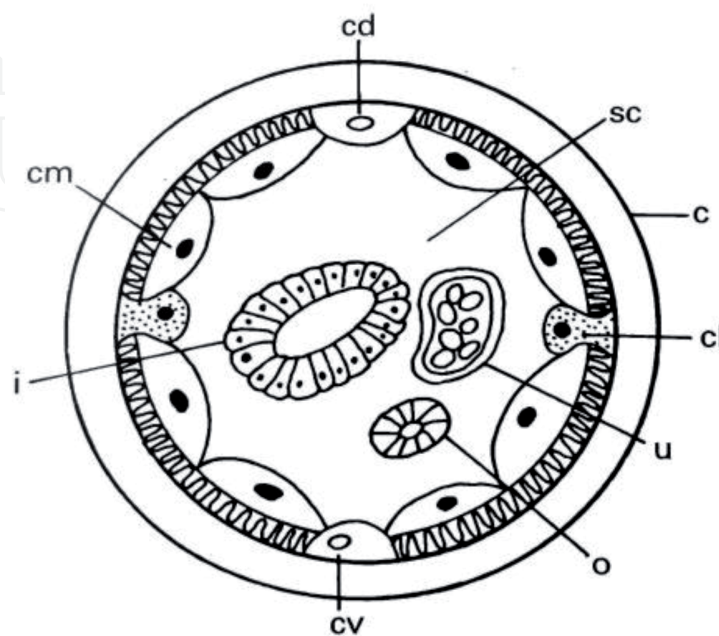


Figure 1.

Nematode cross section (c) cuticle, (cd) dorsal field, (cl) lateral field, (cm) muscle cell, (cv) ventral field, (i) intestine, (o) ovary, (u) uterus.

anus, the copulatory muscles, those of the copulatory bursa, of the spicules, of the gubernaculum and vulva [4].

Cordero del Campillo *et al.* [6] described the anatomy of nematodes in detail regarding the digestive system of nematodes, indicating that it is elongated and has a sac-like shape; it is composed of different organs (**Figure 2**). The mouth is located in the subdorsal or ventral apex, and the primitive model is made up of six lips with two papillae each distributed in two circles (internal and middle) and a third or external circle of four papillae and two lateral amphids, although there are extensive variations in morphology and position. The oral cavity or orifice is a dilation in which hooks or teeth are found both in the cuticular walls in the oral capsule or in the bottom of the cavity. The oesophagus is a radiated muscular organ covered by a thick cuticle, and the muscles that occupy the oesophageal lumen contain three glands, which produce enzymes for its digestive function (**Figure 3**). The intestine

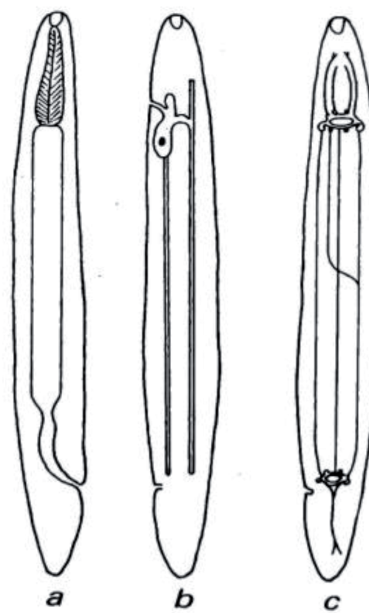


Figure 2.
Schematization of (a) digestive system, (b) excretory system, (c) nervous system.

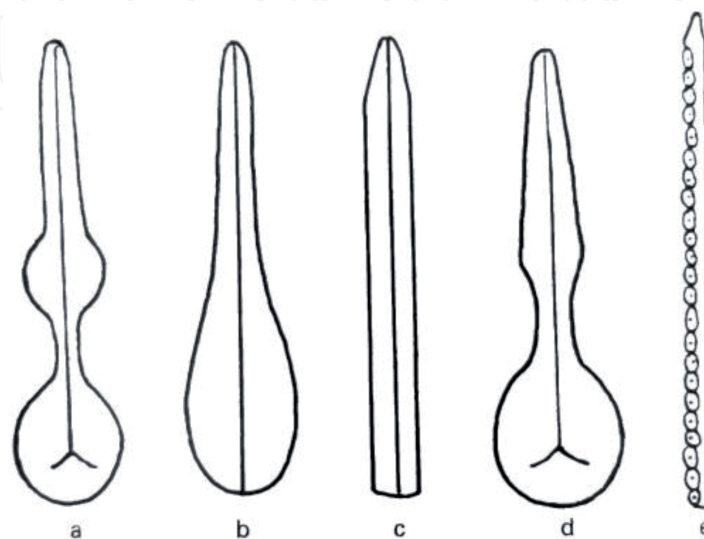


Figure 3.
Different types of nematode oesophagus; (a) rhabditoid, (b) strongyloid, (c) filariform, (d) oxyuride, (e) trichuroid.

is cylindrical in shape and is composed of a basal lamina and a single cell epithelium with a nonmuscular wall covered by microvilli (**Figure 2**). Finally, the rectum is found as an invagination of the cuticular lining that in males gives rise to the cloaca; in some species, it also has glands. In addition to the functions of absorption and defecation of the rectum, it fulfils other functions as an organ of secretion.

The excretory system contains two unbranched lateral tubes that are part of the lateral cords of the hypodermis. The excretory duct comprises the transverse canal to the excretory pore, which generally encompasses the cephalic and cervical region of the nematode.

The nervous system of nematodes is formed by the circumoesophageal ring, which contains ganglia and surrounds the oesophagus, with numerous longitudinal nerves (**Figure 2**). On the dorsal and ventral lines of the body of the nematodes are two of the six short nerves that pass through the anterior part of the body, and another six long nerves pass to the posterior end of the body, likewise a shorter nerve in the ventral line, and the longest in the dorsal line and two small ones in each lateral line. The main nerves emit nerve fibres that can become entangled throughout the body and rejoin these nerves or follow other routes. In general, the sensory organs of parasitic nematodes are less developed than those of nonparasitic nematodes since parasitic life degenerates and atrophies the sensory organs. For these, if we can find the oral papillae, a pair of cervical papillae, and amphidal papillae located in the anterior end, in the posterior end of the body of the male, we can find the genital papillae and other called phasmids in the posterior end of males and females, we can also find an anal ganglion.

The reproductive organs of nematodes are filiform, whitish, long and spirally wound, and their apical end is blind. They continue with long tubes of similar morphology that lead them to the genital cells to the outside. Both the ovaries and the testicles begin as thin threads and are transformed into a central cord, and the surrounding genital cells are later transformed into genital ducts. The male has only one testicle and a vas deferens through which sperm discharge, a seminal vesicle where sperm are stored, and an ejaculatory duct that ends in the cloaca. The testes of most nematodes are of the telegonic type; we also found spermatogonia that extend from the distal portion of the tube and complete along the walls of the central rachis. The spicules are the copulatory organs that are generally found in nematodes, which are elongated and filiform organs of varied dimensions. The spicules are formed in the dorsal sac of the cloaca and are formed by cuticular material. The retractor and ejector muscles are responsible for supporting the spicules, and the gubernaculum is the accessory organ on which the spicules slide and are oriented into and out of the cloaca. Some species also have a caudal pouch, which helps the male attach himself onto the female, and those that do not have it have other cuticular structures, grooves and rough areas.

The female genital tract is formed by the ovary that goes through a maturation process where the oogonia begin in the germinal zone and end in the maturation zone. The oviduct is a short and narrow tube through which the oocytes pass, containing epithelial cells at its base. The seminal receptacle is widening at the beginning of the uterus where sperm are stored. The uterus has an epithelial layer, a basal lamina and muscular annular cells. The vagina is covered in its proximal part by a cuticle, and the vaginal opening is in the ventral medial line of the helminth. The reproductive system of females can be monodelphic, didelphic or polydelphic.

The eggs of the nematodes have a more or less oval shape, and depending on the species, they also vary in size and content. Generally, we find three layers: the lipid layer, the chitinous layer and the external or vitelline layer.

3. Life cycle

Generally, parasitic nematodes are sexually reproduced; males produce sperm, and females produce ovules, which are fertilised after copulation. Embryonic development includes the stages of morula, blastula, gastrula, and tadpole where the embryo acquires its shape. The life cycle includes an egg stage, larval stages (three or four) and an adult stage. Between each larval stage, there is a moulting or change in the cuticle, which can be rigid or elastic and allow growth. Through enzymatic action, each larval stage is released from its envelope to reach the next stage, which may be preceded by lethargy. The life cycles may or may not have one or more intermediate hosts, and the eggs or larvae produced in the definitive host are not infectious, except for rare exceptions. Larval development need to reach the infective stage. In the direct cycles, this development occurs in wet soil, prairies or water. In the indirect cycles, the development up to the infective stage occurs in the intermediate host. In the direct cycle, infestation is usually orally through the ingestion of eggs or larvae; and in the indirect cycle, it can be orally through the ingestion of the intermediate host or arthropod bites [5].

Larval migration to reach the site where they reach sexual maturity can occur through the digestive, hepato-cardio-pulmonary or lymphatic-cardio-pulmonary tracts. The process in which a developmental stage reaches another host includes a complex system of relationships between the animal population and the environment, which vary in time and space. The influence of the environment is important with factors such as temperature, humidity, luminosity, winds, rainfall, types of soil, types of vegetation and seasonal variation. Direct sun rays and dehydration rapidly destroy the larval stages, and the temperature has a range in which the conditions are optimal; outside this range, the physiological process stops and can be destroyed [7].

4. Entomopathogenic nematodes

The entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae are obligate parasites of arthropods, which live in the soil and are ubiquitous and are used commercially to suppress insect pests that live in the soil in agricultural fields [8]. Their use is incipient in the veterinary field.

Nematodes of the Steinernematidae family are characterised by their mutualistic association with bacteria of the genus *Xenorhabdus*. This family is currently comprised by two genera, Steinernema, with more than 70 species, and Neosteinerema, with a single species, *N. longicurvicauda* [1].

The family Heterorhabditidae consists of one genus, *Heterorhabditis*, with *H. bacteriophora* as the model and 17 other species described. These have a life cycle similar to that of nematodes in general, but the adults that result from infectious juveniles (IJs) are hermaphrodites. The eggs laid by the hermaphrodites produce juveniles that become males and females or IJ. Males and females mate and produce eggs that develop in IJ [1].

Natural entomopathogenic nematodes can suppress insects in a wide variety of ecosystems. Insects at any stage of life that come into contact with infested soil are potentially susceptible to infection, and persistent populations of entomopathogenic nematodes in agricultural systems can provide valuable assistance to producers by reducing the costs associated with the management of insect pests. Knowing the environments in which entomopathogenic nematodes persist successfully helps to conserve the natural populations of these insect pathogens that are potentially valuable for agricultural production [8].

4.1 Isolation of entomopathogenic nematodes

Because entomopathogenic nematodes are found in the rhizosphere, moist soil must be sampled for their isolation; it must be sieved to keep it free of organic matter and placed in containers adding larvae of the last instar of greater wax moth *Galleria mellonella* (Linnaeus), which has the objective of being a trap insect to complete the isolation. *G. mellonella* larvae are susceptible to infection by entomopathogenic nematode species [4].

The container with the soil and the larvae of *G. mellonella* is covered and inverted and incubated at $25 \pm 1^\circ\text{C}$ for seven days. After this time, the larvae and/or pupae are recovered and placed in Petri dishes with a double layer of wet filter paper to maintain a high relative humidity and favour the development of the infection [9–10].

Once nematode infection is observed on the dead larvae, it is transferred to a White trap, which helps to separate infectious juvenile nematodes [11]. With the result of this isolation, they reproduce again in other larvae of *G. mellonella* to make their identification and later use them for the necessary assessments.

5. Anthelmintics

Anthelmintics are drugs used in the treatment against parasitic diseases, and they continue to be the cornerstone of parasite control programmes in animals; however, their irrational use has led parasites to develop resistance [12].

Since the parasites are grouped into three categories, Nematoda, Cestoda and Trematoda, there are also three categories or groups of drugs that are available for their treatment. Nematocidal drugs against intestinal worms, hookworms, *Ascaris* and *Strongyloides* include piperazine, mebendazole, thiabendazole, pyrantel, ivermectin and diethylcarbamazine, among others. Antitrematodal drugs include praziquantel, bithionol sulfoxide, oxfamiquine, and metrifonate. The third group of antihelminths are anticestodal drugs, such as niclosamide, which are applied against *Taenia*, *Echinococcus* and *Diphyllobothrium*. Levamisole is often prescribed as an anti-parasitic drug against nematodes such as *Ascaris* and tricostrongyloid species [13].

The most commonly used anthelmintics, their mode of action and mechanism of excretion to the environment are listed in **Table 1**.

5.1 Mechanisms of excretion of some chemicals used as anthelmintics

Because there is a diversity of anthelmintics, only some of the currently most used will be described as an example of the studies that have been performed for each of the active ingredients available.

There are several pharmacokinetic cycles for anthelmintics in animals, from which it is derived that excretion varies; for example, thiabendazole follows an enterohepatic cycle, the amount that is absorbed is rapidly metabolised in the liver by hydroxylation, and its main metabolite is 5-hydroxythiabendazole, which is also metabolised by glucuronidation and sulfate formation. After 8 h of its administration, 90% of the drug is eliminated as a metabolite through the urine and 5% in the faeces. Five days after the last dosage, it was completely eliminated from the body [13].

The fenbendazole that is absorbed is metabolised (and vice versa) and converted into oxfendazole (active compound), fenbendazole sulfone, fenbendazole-2-amino-sulfone and other minor metabolites. The drug that is not absorbed (most of it) is eliminated in faeces and the rest in urine and milk, where 0.3% of the applied dose is detected. In sheep, cattle, and pigs, 44 to 50% of the fenbendazole dose is excreted unchanged in the faeces and less than 1% in urine [32, 33].

Chemical group	Drug	Target/mode of action	Main mechanism of excretion
Aminoacetonitriles	Monepantel*	nAChR allosteric modulator	Urine and feces [14, 15]
Benzimidazoles	Albendazole* Cambendazole Fenbendazole Mebendazole* Oxfendazole Oxibendazole Parbendazole Thiabendazole* Triclabendazole	β -Tubulin inhibitor Fumarate reductase inhibitor β -Tubulin inhibitor	Urine and feces [14, 15]
Benzimidazoles (pro-)	Febantel Netobimin Thiophanate	β -Tubulin inhibitor	Urine and feces [16, 17]
Cyclooctadepsipeptides	Emodepside	LAT-1/SLO-1 inhibitor	Urine and feces [18]
Imidazothiazoles	Tetramisole* Levamisole*	L-subtype nAChR agonist	Urine [19, 20]
Macrocyclic lactones	Abamectin* Doramectin Ivermectin* Moxidectin* Nemadectin	Glutamate- and GABA-gated chloride channels receptor agonist	Urine and feces [21–23]
Organophosphates	Dichlorvos Haloxon Trichlorfon	Acetylcholinesterase inhibitor	Urine [24]
Pyrazinoisoquinolines	Phenothiazine Piperazine Praziquantel	GABA receptor agonist Depolarization of the tegument. Rapid levels of Ca ²⁺ in the sarcoplasmic reticulum	Urine [25, 26]
Salicylanilides	Closantel Niclosamide Oxyclozanide Rafoxanide	Uncoupler of the oxidative phosphorylation	Urine and feces [23, 27]
Spiroindoles	Derquantel*	nAChR antagonist	Urine [28]
Substituted phenols	Bithionol Nitroscanate Nitroxynil	Uncoupler of the oxidative phosphorylation	Urine and feces [18]
Tetrahydro-pyrimidines	Morantel* Pyrantel pamoate* Pyrantel tartrate	L-subtype nAChR agonist	Urine and feces [23, 29, 30]

Note: The compounds which are marked with an asterisk are also used in humans.

Abbreviations: GABA, γ -aminobutyric acid; LAT-1, latrophilin-1; nAChR, nicotinic acetylcholine receptor; SLO-1, slowpoke potassium channel type 1.

Table adapted from Sepúlveda-Crespo et al. [31].

Table 1.

Anthelmintics most commonly used for treatment in humans and veterinary medicine, mode of action and mechanisms of excretion to the environment.

For closantel, its elimination is up to 75% in faeces and to a lesser amount in urine 0.57, 50% of the administered dose is eliminated in 50 to 85 hours, excreting up to 90% of the dose unchanged [27, 34].

Regarding the complete cycle of pharmacokinetics in animals, ivermectin, for example, is a commonly used drug that has been developed by laboratories for its application by different routes (subcutaneous, oral and topical). The oral route shows lower bioavailability, but its values in plasma can last from seven to 14 days, so in low doses (10–40 µg/kg/day), it can be very effective for the control of infestations by parasites (the intramuscular route is not recommended). The absorption processes show differences according to the routes of application and the species treated. Some oily preparations applied subcutaneously reach therapeutic concentrations of 80 to 90 days with a half-life of 36 hours [35]. It has a high volume of distribution with slight variations between species. Because it is a natural lipophilic substance, it is widely distributed in all tissues and tends to accumulate in adipose tissue. The highest concentrations are found in the liver, bile and skin, while the lowest concentrations are in the brain. It is poorly metabolised in the body; therefore, a large part of the dose is excreted unchanged [22].

It has been detected that the gastric content has the highest concentration of the drug. On the other hand, it is concentrated in large amounts in the mucus and intestinal content, so it is feasible to recover a large amount in the faeces, regardless of its route of administration. Ivermectin metabolism is carried out by hydroxylation processes in the rumen, stomach or intestine, regardless of the route of administration. Its metabolites are 24-hydroxymethyl-H2B1a, and 24-hydroxymethyl-H2B1b is eliminated by bile, so large amounts are detected in faeces, although it is also excreted in urine (2%) and in milk. Faecal excretion represents 90% of the total administered dose and in cattle up to 98% or more [35].

In horses, unlike ruminants, the absorption process is faster after oral administration than with subcutaneous administration, and although the injection results in a greater bioavailability, the oral route is preferred, since parenteral administration can produce local swelling and other adverse reactions. Plasma concentrations are higher and are reached more quickly in horses than in sheep, probably because the rumen delays absorption in ruminants. However, the half-lives of sheep in subcutaneous and oral application were 3.7 and 2.8 days, respectively, similar to those of sheep. In horses, the mean residence time is also longer after oral administration (4.2 days) and subcutaneous administration (3 days) and longer in donkeys (6.5 days), with a half-life of 7.4 days. In horses treated subcutaneously, most of the dose (90%) was excreted faecally in 4 days. The higher concentrations found in equine faeces compared to cattle faeces have been attributed to a lower production of more concentrated faeces [22].

Once in the environment, ivermectin can be rapidly degraded when exposed to sunlight. This photodegradation occurs in the presence of ultraviolet light and can occur between 0.5 and 23 days, a period in which it can affect living beings that have contact with the drug, such as earthworms, beetles, insects, fish and even humans [36]. If the meat or by-products of treated animals are consumed by humans, it usually constitutes a public health problem. The residual effect of the drug can be 10 to 12 weeks, and this is considered ideal for the control of ectoparasites, such as fleas, ticks or flies [35].

6. Tests to determine the resistance of parasitic nematodes of animals

6.1 *In vitro*

6.1.1 *Larval motility test*

The test was performed in a flat-bottomed cell culture plate; to facilitate the procedure, a 24-well plate was used. Nematodes are applied, and counts are performed

in each of the wells. Finally, the treatments are inoculated to avoid mortality due to inadequate management [37]. The plate was incubated at $25 \pm 1^\circ\text{C}$ for 24 hours in complete darkness, after which a second nematode count was performed to determine the living and dead individuals in each well [38].

6.1.2 Larval migration inhibition test

For the larval migration inhibition test, a migration system is used that allows the physical separation of IJ with motility of the immobile ones through a $25 \mu\text{m}$ polypropylene mesh filter. The diameter of the pores allows active larvae, but not dead larvae, to pass through the mesh. In a new plate, the mesh filter is placed in each well, and the total volume of each well is transferred from the plate used in the motility test. The samples are incubated for 24 hours at $25 \pm 1^\circ\text{C}$ in complete darkness, and then the live and dead nematodes were counted [39].

6.2 *In situ*

6.2.1 Faecal egg count reduction

The egg counts of parasitic nematodes present in the excrement are considered the main test for parasite control because it has been shown that animals maintain relatively consistent levels of egg excretion over time.

In this technique, a suspension of faecal material is dispersed in a solution of higher density than the eggs of parasites (solution with common salt, 33% zinc sulfate, 35% magnesium sulfate, saturated sugar solution, sodium nitrate, etc.). The difference in specific gravity causes the eggs to rise to the surface or all float to the same level. The solution mixed with the excrement is allowed to settle, and most of the faecal particles will fall towards the bottom since their density is greater than that of the solution. This step is important for some parasitological diagnostic techniques but not for this test. Therefore, the procedure should be completed in the shortest possible time or regularly homogenise the mixture.

To achieve the procedure and determine the reduction of eggs per gram of excreted faeces, it is necessary to use the parasitological technique that uses the device called McMaster chamber where the number of eggs in a given amount of liquid (0.15 mL) is verified and then procedures to estimate the amount of parasitic nematode eggs per gram of excrement used initially are performed, this activity must be done before and after treatments with anthelmintics, and subsequently calculate the percentage of reduction of the egg count with the following formula:

$$FECR = \left(\frac{(EPG \text{ pretreatment} - EPG \text{ posttreatment})}{EPG \text{ pretreatment}} \right) \times 100 \quad (1)$$

This technique is commonly used in the initial tests when populations of chemically resistant nematodes are suspected.

7. Biological models used for the assessment of anthelmintics

There are many limitations to conducting experimental studies on parasitic nematodes to assess the anthelmintic potential of a new product or drug, both for *in vitro* studies and *in vivo* studies. The difficulties, among others, are the difficulty of

evaluating their activity in adult stages of parasitic nematodes kept outside the host, the cost of infection and sacrifice of experimental donor animals, and the impossibility of obtaining large quantities of the stages of the nematodes under study, the cost of maintaining the hosts, labour, the total time of handling the animals, and the approval of different ethics committees, among others not listed [40–42].

Although there are problems because *in vitro* studies with biological models different from parasitic nematodes sometimes do not offer reliable results, the use of entomopathogenic nematodes can help to standardise studies or preliminary tests that allow establishing the correct methodology, as its use to determine the effect on nontarget species.

Of the nematodes commonly used as a biological model for the assessment of anthelmintic products *in vitro* is *Caenorhabditis elegans*, and *in vivo* assessments have used rodents when the parasite allows it [43–45]. It is essential to consider that both physical and biological differences between nematode species can be a limitation for the assessment of new drugs developed to inhibit specific parasitic stages; therefore, this characteristic should be considered when using a nontarget species. (*C. elegans*, entomopathogenic nematodes, among others) for assessments. Currently, new tests or more specific tests are being published to mitigate the deficiency of not using the target species.

8. Nematocide assessment techniques on phytopathogenic nematodes

Nematocides are usually toxic with a broad spectrum and have high volatility or other properties that promote migration through the soil. The use of chemical nematocides is increasing every day even though they have been banned [46] or even though alternative nematocides are being created [47, 48].

Despite the diversity of methodologies used in the assessment of nematocides, the results are differences between assessed compounds, and the same assessment must be performed several times; sometimes, the limited number of nematodes cultured in laboratory conditions does not allow for the necessary repetitions. In addition, the standardisation time of tests can be prolonged.

There is a diversity in the assessment methodologies of phytopathogenic nematocides. From petri dish assessments with the challenge of the chemical being in relatively large spaces, which allow the nematode much movement [49], to the assessment in cell culture chambers [50] with an incubation and assessment period similar to those carried out in studies on parasitic nematodes of animals [51].

Due to the characteristics of entomopathogenic nematodes, they will always be a biological model with great availability to establish an assessment technique in a new laboratory, since the availability of specimens allows us to test a bioassay a greater number of times before using phytopathogenic nematodes and thus train the personnel who will carry out the process.

9. Advantages of the possible use of entomopathogenic nematodes as a model for the biological assessment of anthelmintics

As in all tests where biological organisms are used, there are advantages and disadvantages, in this case between the test performed and between the organisms used as a biological model, so the main objective is to strengthen and exploit the advantages. Below are some advantages and disadvantages of the possible use of entomopathogenic nematodes as biological models to determine the effectiveness of a biological or chemical nematocide of parasitic nematodes of plants or animals.

Advantages

- Small size.
- The short life cycle is quickly completed.
- In the right conditions they reproduce all year round.
- Short life span.
- Known and simple anatomy.
- Abundant progeny.
- Simple and economical cultivation.
- It can be maintained for long periods in the laboratory.
- Several strains can be easily maintained in a small space.
- Constant motility with little stimulus.
- They are cosmopolitan.
- They live in the rhizosphere in more than one of their stages of the biological cycle.
- Isolation and identification are relatively fast, inexpensive and do not require much training.

Disadvantages

- Relatively simple anatomy.
- Possible problem due to the type of feeding in its different stages of development.
- It is not possible to assess bioavailability and organic toxicity.
- It could only be used for standardisation of tests.
- The toxicity to entomopathogenic nematodes is probably not similar to that of parasitic nematodes.
- The differences in their anatomy and physiology should be considered in studies.

10. Conclusions

Currently, there is an urgent demand for the development of new anthelmintic drugs due to various circumstances; reaching their generation and assessment is not a short route and requires many economic resources; every time there has been the

need for them, it has been solved. However, the assessment of the product or the new drug in controlled or field conditions is extremely complicated.

Research is required for continuous improvement in the management of parasitic nematodes, emphasising the reduction of the use of chemotherapeutics and the development of resistance to anthelmintics, for which viable options are required for the assessment of this resistance. Entomopathogenic nematodes offer an opportunity that favours these aspects, in addition to helping to understand the interactions of these chemicals with the rhizosphere and the environment in general once they are excreted by animals.

Complications in the assessment of new drugs can be analysed in various ways; however, this chapter proposes an alternative solution for the lack of target nematodes (human or animal parasites) in sufficient quantity and in the biological stage of the nematodes in which the assessment is desired. Entomopathogenic nematodes, due to their characteristics, are an alternative to perform the assessments of new drugs on nontarget nematodes that even allow generating populations resistant to a chemical product to assess a new drug, combinations of them or to simulate activities that limit dispersion of the resistance.

Conflict of interest

The authors declare no conflict of interest.

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