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# NEMATODES AS BIOCONTROL AGENTS OF INSECT PESTS OF PLANTS

*[1]Edelduok, E. G. and [2]Umana, E. J.
1. Department of Biological Sciences, Akwa Ibom State University, Ikot Akpaden, Mkpat Enin, P. M. B. 1167, Uyo, Akwa Ibom State, Nigeria
2. Department of Botany, University of Calabar, Calabar
P. M. B. 1115, Calabar, Cross River State, Nigeria
*Corresponding Author's E-mail: e.edelduok@yahoo.com

## ABSTRACT

Nematodes are microscopic, non-segmented, elongated roundworms. Some nematodes are harmful, being pests that can destroy crops both on the field and in storage. Conversely, other nematodes may be beneficial to human in that they could be employed as biocontrol agents to checkmate pests of crops. Biological control is usually preferred to other methods of pest control because it is safe and most times, does not destroy beneficial organisms. Since beneficial nematodes are non-harzardous to the environment, there has been a growing interest in the use of these beneficial nematodes to control insect pests of plants. One important group of beneficial nematodes used for the control of insect pests of plants is the insect-pathogenic or entomopathogenic nematodes (EPNs). However, the use of EPNs is still in its infancy and there remains a lot of potentials waiting to be fully harnessed. Thus, this paper examines the use of entomopathogenic nematodes of plants as a complimentary means of managing insect infestation.

**Keywords:** Biocontrol agent, entomopathogenic nematodes, insect pests, plants

## 1. INTRODUCTION

Nematodes are microscopic, non-segmented, elongated roundworms which are colourless and without appendages (Miles *et. al.*, 2012). They belong to the phylum, Nematoda. Some nematodes are beneficial while others are not. Non-beneficial nematodes are also called 'plant-parasitic nematodes' and cause damage to crops and other types of plants. Beneficial nematodes on the other hand attack soil-borne insect pests, mostly sterilizing or otherwise debilitating their hosts and yet are not harmful to humans, animals or plants, and can therefore be used as biological control agents (Denno *et. al.*, 2008). Biological control could be defined as the decline in pest density as a result of the presence of natural enemies (Dreves *et. al.*, 2013). Biological control is preferred to other methods of control because it is safe, non-hazardous and does not destroy beneficial organisms. For a living organism to be exploited for the biological control of pests, there are certain attributes it must possess: it must be host specific, parasitism must be lethal, it must be easily manipulated in the laboratory, it must be easily mass produced, it must be easily disseminated with standard equipment, and it must not be harmful to the environment (Grewal *et. al.*, 2005). One group of beneficial nematodes used in controlling insect pests of plants is the insect-pathogenic or entomopathogenic nematodes (EPNs). Insect-pathogenic, or entomopathogenic, nematodes (EPNs) are a group of soil-dwelling roundworms which only kill insects that live in, on, or near the soil surface, usually closely associated with plants (Dreves, 2013). These nematodes occur naturally in soil and are found in most places where plants grow. They possess the desirable attributes needed for a biological agent to be employed in biological control. This paper discusses the use of entomopathogenic nematodes for the control of insect pests of plants.

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## 2. Entomopathogenic nematodes

Nematodes that parasitize insects, known as entomopathogenic nematodes (EPNs), have been described from 23 nematode families (Koppenhofer, 2007). Of all of the nematodes studied for biological control of insects, nematodes belonging to the families Steinernematidae and Heterorhabditidae have received the most attention because they possess many of the attributes of effective biological control agents (Kaya and Gaugler, 1993; Grewal

*et. al.*, 2005; Koppenhofer, 2007). They can be easily mass produced, have narrow host specificity against pests, have no known negative effect on the environment, are safe to use in sensitive areas, have a broad insect host range, are safe to plants and vertebrates, are commercially available, are not phytotoxic, and can debilitate or kill insects within 48 hours (Poinar, 1990; Berry *et. al.*, 1997). In some cases, these EPNs can be applied in conjunction with insecticides without losing their infectivity (Kaya and Burlando, 1989; Nishimatsu and Jackson, 1998), and can be applied in the field with equipment that is typically used for synthetic chemical pesticides (Georgis, 1990). In addition, EPNs have the potential to reproduce in soil environments, and are capable of maintaining an efficacious population density for at least one additional season after application (Shanks and Agudelo-Silva, 1990).

The infective juvenile of an EPN is more or less microscopic, anything from 0.5 mm to 1.5 mm long depending on species. It has a closed mouth and anus and cannot feed until it finds an insect. Usually it is found in soil, is activated by insect movement and then follows a gradient of carbon dioxide to find the insect. It needs to get into the insect's blood cavity in order to kill it. EPNs enter through the insect's natural body openings such as the mouth, anus or respiratory inlets (spiracles) and then penetrate into the blood cavity from the gut or breeding tubes (Poinar, 1990). Heterorhabditis species can also penetrate through chinks in the insect's armour (the interskeletal membranes) by scratching away at these with a special tooth (Bedding and Molyneux, 1983).

Once in the insect's blood the EPN infective juvenile releases a highly specialized symbiotic bacterium found only in EPNs (*Xenorhabdus* spp. in Steinernema, and *Photorhabdus* spp. in Heterorhabditis). These symbiotic bacteria multiply and rapidly kill the insect within a day or two. The bacteria then convert the insect into suitable food for the nematodes and produce a range of antibiotics and anti-feedants that preserve the dead insect while the nematodes feed and breed up within it. After about 10 days a medium-sized scarab cadaver (insect corpse) may produce up to 100,000 or more infective juvenile EPNs that are released into the soil and seek out new insect pest hosts. Unfortunately there are many fungi and other organisms that can attack the infective juveniles before they are able to enter an insect pest (Kopenhofer *et. al.*, 1996; Kopenhofer *et. al.*, 1997).

Entomopathogenic nematodes are extraordinarily lethal to many important insect pests, yet are safe for plants and animals. This high degree of uninfectivity to humans means that unlike chemicals, nematode applications do not require masks or other safety equipment; and re-entry time, residues, groundwater contamination, chemical trespass, and pollinators are not issues. Most biocontrol agents require days or weeks to kill, yet nematodes, working with their symbiotic bacteria, can kill insects within 24 - 48 hours. Dozens of different insect pests are susceptible to infection, yet no adverse effects have been shown against beneficial insects or other non-target organisms in field studies (Georgis *et. al.*, 1991; Akhurst and Smith, 2002). Nematodes are amenable to mass production and do not require specialized application equipment as they are compatible with standard agrochemical equipment, including various sprayers (e.g., backpack, pressurized, mist, electrostatic, fan, and aerial) and irrigation systems.

Even though EPNs possess these desirable attributes, their successes as biological control agents are particularly enhanced by understanding their life cycles and functions, matching the correct nematode species with the pest species, applying them during appropriate environmental conditions (soil temperature, soil moisture, sunlight) and applying them only with compatible pesticides.

### 2.1 Life cycle of entomopathogenic nematodes

The life cycle of most nematodes includes an egg stage, four juvenile stages, and an adult stage. The third juvenile stage of EPNs is referred to as the 'infective juvenile' and is the only free-living stage. The non-feeding, developmentally arrested infective juveniles seek out insect hosts and initiate infections. They are capable of surviving in the soil where they are found, and where they attack and infect pest insects (Poinar, 1990). When a host has been located, the nematodes penetrate into the insect body cavity, usually via natural body openings (mouth, anus, spiracles) or areas of thin cuticle. Once in the body cavity, a symbiotic bacterium (*Xenorhabdus* for steinernematids, *Photorhabdus* for heterorhabditids) is released from the nematode gut, which multiplies rapidly

and causes rapid insect death. The nematodes feed upon the bacteria and liquefying host, and mature into adults. Steinernematid infective juveniles may become males or females, whereas heterorhabditids develop into self-fertilizing hermaphrodites although subsequent generations within a host produce males and females as well. The life cycle is completed in a few days, and hundreds of thousands of new infective juveniles emerge in search of fresh hosts. Under optimal conditions, it takes 3 – 7 days for steinernematids and heterorhabditids to complete one life cycle from egg to egg inside a host. Emergence of infective juveniles from the host requires about 6 – 11 days for steinernematids and 12 – 14 days for heterorhabditids (Kaya and Koppenhofer, 1999).

## 3. Production and storage of entomopathogenic nematodes

Entomopathogenic nematodes can be mass produced for use as biopesticides using *in vivo* or *in vitro* techniques (Shapiro-Ilan and Gaugler, 2002). *In vivo* technique (culture in live insect hosts) involves production and application of nematodes in infected host cadavers; the cadavers (with nematodes developing inside) are distributed directly to the target site and pest suppression is subsequently achieved by the infective juveniles that emerge. Conversely, *in vitro* technique involves culturing the nematodes in solid culture (e.g. growing the nematodes on crumbled polyurethane foam), or in liquid culture. In *in vitro* solid culture application technique, cultures of the nematode-bacterium complex are pre-incubated with the symbiotic bacteria before the nematodes are inoculated (Ehlers, 2001). Once the infective juveniles of EPNs emerge, they are harvested and applied to plants using various spray equipment and standard irrigation systems (Shapiro-Ilan *et. al.*, 2012). In *in vitro* liquid culture application technique, symbiotic bacteria are also first introduced followed by the nematodes. Various ingredients used for liquid culture media include soy flour, yeast extract, canola oil, corn oil, thistle oil, egg yolk, casein peptone, milk powder, liver extract and cholesterol among others (Surrey and Davies, 1996; Shapiro-Ilan *et. al.*, 2012). Once the culture is completed, nematodes can be harvested from media via centrifugation (Surrey and Davies, 1996).

A variety of formulations have been developed to facilitate nematode storage and application including activated charcoal, alginate and polyacrylamide gels, baits, clay, paste, peat, polyurethane sponge, vermiculite, and water-dispersible granules. Depending on the formulation and nematode species, successful storage under refrigeration ranges from one to seven months. Optimum storage temperature for formulated biocontrol agents varies according to species. Generally, steinernematids tend to store best at 4 - 8 °C whereas heterorhabditids persist better at 10 - 15 °C.

## 4. Application and factors militating against the efficacy of entomopathogenic nematodes as biocontrol agents

Nematodes are formulated and applied as infective juveniles, the only free-living and therefore environmentally tolerant stage. Technological advances in nematode production, formulation, quality control, application timing and delivery, and particularly in selecting optimal target habitats and target pests, have increased the efficacy of nematode agents. Nematodes have consequently demonstrated efficacy in a number of agricultural and horticultural market segments.

Even though EPNs are useful against many soil insect pests in diverse cropping systems, they are not fully utilized. Like other biological control agents, nematodes are constrained by being living organisms that require specific conditions to be effective. Thus, desiccation or ultraviolet light rapidly inactivates insecticidal nematodes; chemical insecticides are less constrained. Similarly, nematodes are effective within a narrower temperature range (generally between 20 °C and 30 °C) than chemicals, and are more impacted by suboptimal soil type, thatch depth, and irrigation frequency (Georgis and Gaugler, 1991; Shapiro-Ilan *et. al.*, 2006). Nematode-based insecticides may be inactivated if stored in hot vehicles, cannot be left in spray tanks for long periods, and are incompatible with several agricultural chemicals. Accelerated implementation of nematodes into Integrated Pest Management (IPM) systems will require users to be more knowledgeable about how to use them effectively.

Therefore, based on the nematodes' biology, applications should be made in a manner that avoids direct sunlight, e.g., early morning or evening applications are often preferable. Soil in the treated area should be kept moist for at least two weeks after applications. Application to above-ground target areas is difficult due to the nematode's sensitivity to desiccation and UV radiation; however, some success against certain above-ground targets has been achieved and recently approaches have been enhanced by improved formulations (Shapiro-Ilan *et. al.*, 2010). In all cases, the nematodes must be applied at a rate that is sufficient to kill the target pest; generally, 250,000 infective juveniles per $m^2$ of treated area is required (Shapiro-Ilan *et. al.*, 2002). Additionally, it is important to match the appropriate nematode species to the particular pest that is being targeted (refer to Table 1).

## 5. Habitat

Steinernematid and heterorhabditid nematodes are exclusively soil organisms. They are ubiquitous, having been isolated from every inhabited continent from a wide range of ecologically diverse soil habitats including cultivated fields, forests, grasslands, deserts, and even ocean beaches. When surveyed, EPNs are recovered from 2 % to 45 % of sites sampled (Hominick, 2002).

## 6. Pests attacked

When considered as a group of nearly 80 species, EPNs are effective against a large number of insect pests (Grewal *et al.*, 2005). Additionally, EPNs have been marketed for control of certain plant parasitic nematodes, though efficacy has been variable depending on species (Lewis and Grewal, 2005). A list of many of the insect pests that are commercially targeted with EPNs is provided in Table 1.

## 7. Mode of action of entomopathogenic nematodes on insect pests

To ensure infection and control, an understanding of host-finding strategies is necessary to properly match EPN species to pest insects (Gaugler, 1999). Only the infective juvenile stage of EPNs survives in the soil, locates and penetrates insect pests. Infective juvenile EPNs locate their hosts in soil through two major ways: ambushing and cruising.

- **Ambushing:** EPNs that use the ambushing strategy tend to remain stationary at or near the soil surface and locate host insects by direct contact (Campbell *et. al.*, 1996). An ambusher (e.g. *Steinernema carpocapsae*, *Steinernema scapterisici*) searches by standing on its tail so that most of its body is in the air, referred to as 'nictation'. The nictating nematode attaches to and attacks passing insect hosts. Ambusher EPNs most effectively control insect pests that are highly mobile at the soil surface, such as cutworms, armyworms, and mole crickets.
- **Cruising:** EPNs that use the cruising strategy are highly mobile and able to move throughout the soil profile. Cruisers (e.g. *Steinernema glaseri, Heterorhabditis bacteriophora*) locate their host by sensing carbon dioxide or other volatiles released by the host. Cruiser EPNs are most effective against sedentary and slow-moving insect pests at various soil depths, such as white grubs and root weevils. Once infective juveniles of EPNs are able to locate a host, they search for an entry point into the host (Figure 1) and infect it (Figure 2).

## 8. Soil conditions

EPNs can die if they are applied to soils that are too dry, too hot, or too cold, or if they are exposed to ultraviolet (UV) light from the sun. Nematodes live in the water-filled spaces, or pores, between soil particles. They need water to move and successfully locate a host, and oxygen to survive. Heavy clay soils hold water well, but may contain too little oxygen, and the small pore space may restrict nematode movement. Sandy soils must be irrigated to maintain the water-filled pores. If applying EPNs after an extended dry period, it is advisable to break the crust of the dry soil with a rake or harrow and irrigate the soil before the application, preferably to a depth of 4 – 6 inches. Plant debris should be removed before the application, as it may prevent the nematodes from reaching the soil surface.

Soil temperatures between 25 °C and 28 °C are ideal for applying all EPN species. In general, soil temperatures greater than 29 °C can decrease the efficacy of some nematode species, while soil temperatures less than 10 °C can immobilize others at the soil surface, causing them to be exposed to UV light that can kill them. The range of soil temperatures that nematode species can survive and infect host insects does vary, however. For example, *S. feltiae* can be effective at 14 °C, while *S. riobrave* can be effective at 35 °C. EPNs should be applied late in the day or on a cool, overcast day when light and temperatures are low. EPN's should be applied to moist soil following either a rainfall or irrigation, and should be lightly irrigated afterward. This helps in washing EPN's into the soil and decreases soil surface temperatures. Over-irrigation should be avoided since saturated soil impedes nematode activity due to lack of oxygen.

**9. Preparation for application**

EPNs should be prepared for field application no earlier than one hour ahead of time. If nematodes are in a liquid suspension, the shipment container should be shaken well and the liquid poured into the application container (e.g., tank, backpack sprayer, or watering can). The shipment container should be rinsed twice with cool water (approximately 60 °F), and the rinse water should be poured into the application container. If nematodes are on a sponge, the sponge should be soaked in one gallon of cool water for 10 minutes, and then the water should be poured into the application container. The sponge should be rinsed several times, pouring the rinse water into the application container after each rinse. If nematodes are in vermiculite, the vermiculite-nematode mixture should be added directly to water in the application container and stirred until dispersed. Once the nematodes have been mixed with water, the mixture should be agitated every five minutes to keep the nematodes in suspension and supplied with oxygen.

**10. Application equipment**

The product label should be read for specific application instructions. EPNs that are formulated with vermiculite may be best applied as a granular product. Other formulations can be applied using standard liquid pesticide, fertilizer, and irrigation equipment with pressures of up to 300 PSI. Electrostatic, fan, pressurized, and mist sprayers can be used. If tanks are agitated through excessive sparging (recirculation of the spray mix), or if the temperature in the tank rises above 86 °F, the nematodes will be damaged. Irrigation systems may also be used for applying most species; however, high-pressure recycling pumping systems are not good delivery systems (Shetlar, 1999).

**11. Compatibility**

Entomopathogenic nematodes are compatible (e.g., may be tank-mixed) with most chemical herbicides and fungicides as well as many insecticides (such as bacterial or fungal products) (Koppenhofer and Grewal, 2005). In fact, in some cases, combinations of chemical agents with nematodes results in synergistic levels of insect mortality. Some chemicals to be used with care or avoided include aldicarb, carbofuran, diazinon, dodine, methomyl, and various nematicides. However, specific interactions can vary based on the nematode and host species and application rates. Furthermore, even when a specific chemical pesticide is not deemed compatible, use of both agents (chemical and nematode) can be implemented by waiting an appropriate interval between applications (e.g., 1 – 2 weeks). Prior to use, compatibility and potential for tank-mixing should be based on manufacturer recommendations. Similarly, entomopathogenic nematodes are also compatible with many though not all biopesticides (Koppenhofer and Grewal, 2005). Nematodes are generally compatible with chemical fertilizers as well as composted manure, though fresh manure can be detrimental. Fresh manure may result in a reduction of nitrogen for plant use because of the accelerated growth of microbes that compete for nitrogen. The accumulation of certain nitrogenous compounds produced during the application of fresh manure, are known to be toxic to nematodes (Nahar *et. al.*, 2006). Hence, fresh manure tend to reduce the availability of plant parasitic nematodes.

**12. CONCLUSION**

The use of nematodes as biocontrol agents of plant pests is both beneficial and effective. However, the use of EPNs is really still in its infancy and a lot of potentials are yet to be fully

harnessed. Consequently, active research aimed at increasing the effectiveness of insect-pathogenic nematodes and making them a cost-effective tool for insect pest management should be intensified.

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**Table 1: Use of nematodes as biological control organisms[1] (Shapiro-Ilan and Gaugler, 2010)**

| Target crops | Common names of pests | Scientific names of pests | **Efficacious Nematodes[2]** |
|---|---|---|---|
| Artichokes | Artichoke plume moth | *Platyptilia carduidactyla* | Sc |
| Vegetables | Army worm | Lepidoptera: Noctuidae | Sc, Sf, Sr |
| Ornamentals | Banana moth | *Opogona sachari* | Hb, Sc |
| Bananas | Banana root borer | *Cosmopolites sordidus* | Sc, Sf, Sg |
| Turf | Billbug | *Sphenophorus* spp. (Coleoptera: Curculionidae) | Hb,Sc |
| Turf, vegetables | Black cutworm | *Agrotis ipsilon* | Sc |
| Berries, ornamentals | Black vine weevil | *Otiorhynchus sulcatus* | Hb, Hd, Hm, Hmeg, Sc, Sg |
| Fruit trees, ornamentals | Borer | *Synanthedon* spp. and other sesiids | Hb, Sc, Sf |
| Home yard, turf | Cat flea | *Ctenocephalides felis* | Sc |
| Citrus, ornamentals | Citrus root weevil | *Pachnaeus* spp. (Coleoptera: Curculionidae) | Sr, Hb |
| Pome fruit | Codling moth | *Cydia pomonella* | Sc, Sf |
| Vegetables | Corn earworm | *Helicoverpa zea* | Sc, Sf, Sr |
| Vegetables | Corn rootworm | *Diabrotica* spp. | Hb, Sc |
| Cranberries | Cranberry girdler | *Chrysoteuchia* topiaria | Sc |
| Turf | Crane fly | Diptera: Tipulidae | Sc |
| Citrus, ornamentals | Diaprepes root weevil | *Diaprepes abbreviatus* | Hb, Sr |
| Mushrooms | Fungus gnat | Diptera: Sciaridae | Sf, Hb |
| Grapes | Grape root borer | *Vitacea polistiformis* | Hz, Hb |
| Iris | Iris borer | *Macronoctua onusta* | Hb, Sc |
| Forest plantings | Large pine weevil | *Hylobius albietis* | Hd, Sc |
| Vegetables, ornamentals | Leaf miner | *Liriomyza* spp. (Diptera: Agromyzidae) | Sc, Sf |
| Turf | Mole cricket | *Scapteriscus* spp. | Sc, Sr, Sscap |
| Nut and fruit trees | Navel orange worm | *Amyelois transitella* | Sc |
| Fruit trees | Plum curculio | *Conotrachelus nenuphar* | Sr |
| Turf, ornamentals | Scarab grub[3] | Coleoptera: Scarabaeidae | Hb, Sc, Sg, Ss, Hz |
| Ornamentals | Shore fly | *Scatella* spp. | Sc, Sf |
| Berries | Strawberry root weevil | *Otiorhynchus ovatus* | Hm |
| Bee hives | Small hive beetle | *Aethina tumida* | Hi, Sr |
| Sweet potato | Sweet potato weevil | *Cylas formicarius* | Hb, Sc, Sf |

[1] Nematodes listed provided at least 75% suppression of these pests in field or greenhouse experiments.
[2] Nematode species are abbreviated as follows: Hb = *Heterorhabditis bacteriophora*, Hd = *H. downesi*, Hi = *H. indica*, Hm = *H. marelata*, Hmeg = *H. megidis*, Hz = *H. zealandica*, Sc = *Steinernema carpocapsae*, Sf = *S. feltiae*, Sg = *S. glaseri*, Sk = *S. kushidai*, Sr = *S. riobrave*, Sscap = *S. scapterisci*, Ss = *S. scarabaei*.
[3] Efficacy against various pest species within this group varies among nematode species.

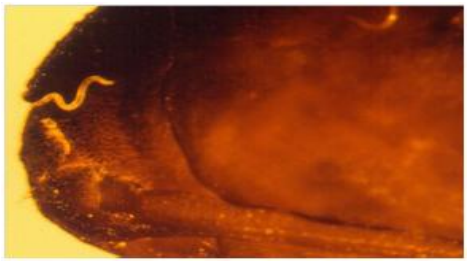

Figure 1: Infective juvenile entomopathogenic nematodes searching for an entry portal into a host (Miles *et. al.*, 2012).

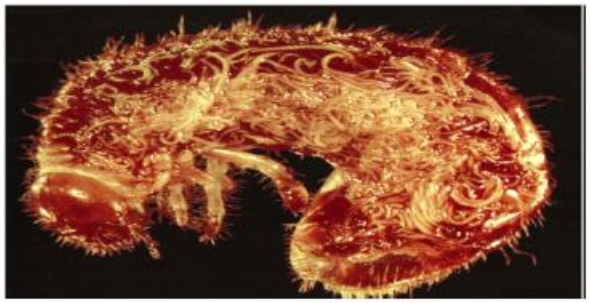

Figure 2: Numerous new entomopathogenic nematodes within a host insect (Miles *et. al.*, 2012).

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