

Roundworms Useful as Biological Control Agents in the Central Rift Valley, Kenya

Shelmith W. Mwaniki
Norman E. Borlaug LEAP Fellow 2006-2007

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In agriculture, nematodes are roundworms that can be either beneficial to plants (by preying on harmful insects) or harmful pests that attack plants. Entomopathogenic nematodes, more specifically, belong to the first group and are insect parasitic nematodes that are useful as biological control agents of insect pests on agricultural crops. Chemical insecticides effectively control insect pests, but they are associated with environmental pollution and operator hazards. Entomopathogenic nematodes, on the other hand, occur naturally in soils and are easy to isolate, culture and apply for insect pest control. Soil factors including soil pH and soil organic carbon may influence nematode function. This study determined entomopathogenic nematode distribution and identity in the Central Rift Valley region of Kenya. A survey for the nematodes was carried out in October 2005, and nematodes were extracted from soils using the greater wax moth as bait. Overall, 30-50% of soil samples yielded nematodes at the rate of 18-71% per agro-ecological zone. Thirty-six percent of sites from cultivated areas and 17% of wild habitat sites tested positive for nematodes. Nematodes occurred at various rates depending on the percent of soil carbon, soil pH, and sea level of the study sites. Thirty-three nematode isolates were collected from soils of the Rift Valley region of Kenya, resulting in the identification of six distinct nematodes. Four of these nematodes were reported in Kenya for the first time, and one was confirmed as new.

Background

The agricultural sector contributes to 25% of the gross domestic product (GDP) in Kenya and offers employment to more than 80% of the population, either in direct farming activities or in agricultural related sectors of the economy. Small-scale farms represent 80% of crop production, most of which is used for home consumption, while excess is sold to generate income. Farmers face several constraints ranging from policy to production and marketing. Production constraints include pests and diseases, poor agronomical practices, low soil fertility, poor farm management and lack of access to more efficient technologies, poor storage, and lack of credit. Marketing constraints may include inaccessibility to marketing information and infrastructure and high costs of inputs, particularly pesticides. Pesticides are also associated with operator hazards, environmental pollution and increased pest resistance. Safer methods of pest control that are accessible to farmers are ideal. Such methods include the use of biological control agents (natural enemies, parasites and parasitoids, and insect pathogens) and the use of botanical extracts and pheromones. Entomopathogenic nematodes are insect parasitic nematodes that can be developed into biological control agents of insects and used as bio-pesticides on farms.

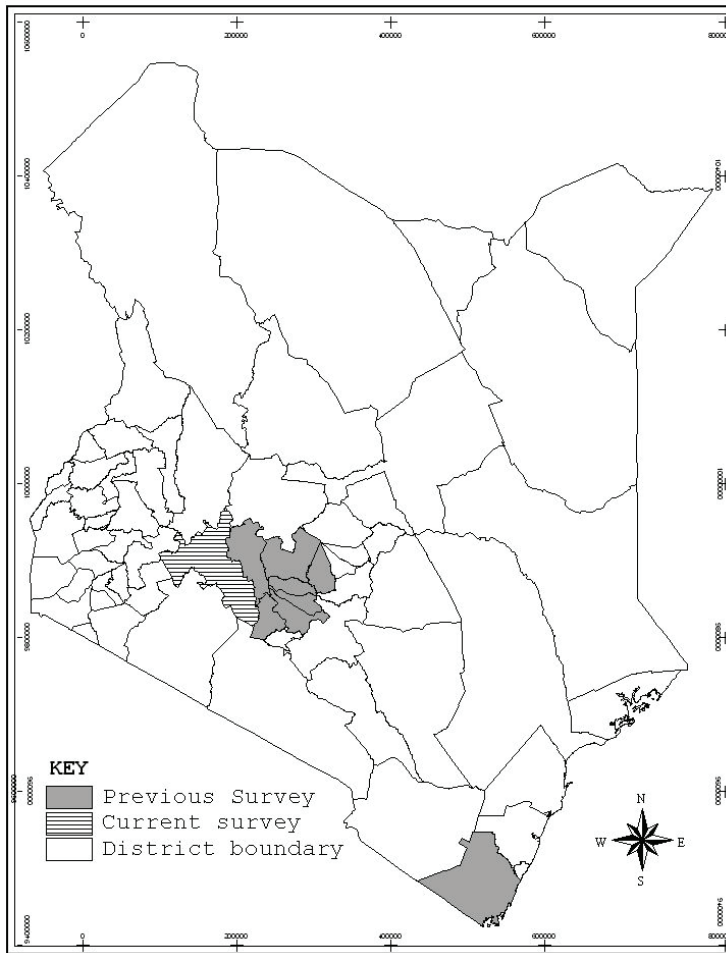
Entomopathogenic nematodes are used for the biological control of arthropod pests (insects and arachnids, among

others) on the basis that they work mutually with bacteria in the family of Enterobacteriaceae. The nematode acts as a carrier for the bacteria to reach the insect gut, while the bacteria recondition the gut to the right environment for nematode reproduction. The host dies in 24-48 hours.

Entomopathogenic nematodes are not harmful to vertebrates and plants. The nematodes reduce pest populations in the natural environment, but their effects become significant when they are applied for the control of specific insect pests. The most commonly researched species belong to two genera, *Steinernema* and *Heterorhabditis*. Numerous species have been isolated, namely those from America and Europe, but the search has extended to be worldwide. Research reports on nematodes from the African continent are mainly from Egypt and South Africa.

Prior to this study, the main work done in Kenya on entomopathogenic nematode identification was a survey carried out in the Central and Coastal lowlands where researchers reported *Heterorhabditis bacteriophora*, *Heterorhabditis indica*, and *Steinernema kari*, a new species. The study described in this brief focuses on expanding the area covered by the previous investigation to widen the genetic scope of entomopathogenic nematodes from Kenya. The study objective is to help

Figure 1. Entomopathogenic nematode surveys in Kenya.



incorporate indigenous entomopathogenic nematodes into farming systems to provide insect pest control and reduce the use of chemical insecticides that are not cost-effective for smallholders and can be harmful to the environment and humans.

Major findings

Thirty-three nematode isolates were collected from soils of the Rift Valley region of Kenya, resulting in the identification of six distinct nematodes. Four of these nematodes were reported in Kenya for the first time, and one was confirmed as new. The isolates were recovered from all samples at a rate of 50%. Specifically, however, researchers found nematodes more frequently in farmland samples than in those from natural habitats. Most of the nematodes found in this study belong to the genus *Steinernema* and were found in soils of 2-3% carbon and with a pH 5-6. More nematodes were isolated from 2400-2600 meters above sea level. Three-quarters of samples from the shores of lakes and dams tested positive for nematodes from the genera *Steinernema* and *Heterorhabditis*. *Steinernema yirgalemense* occurred most widely in sites at 1800 and 2630 meters above sea level, from upper to lower highlands and upper midland zones.

The six nematodes found in this study have improved the genetic base of Kenyan entomopathogenic nematodes and are available for research and as naturally occurring biological control agents at the farm level. The 50% nematode recovery from the region shows that the region was very suitable for nematode survival. Results from the past survey identifying three species (Figure 1) support the current evidence, whereby nematodes belonging to one genus (*Steinernema*) were more frequent than those of the other (*Heterorhabditis*). Results from both studies, however, suggest that the method of isolating nematodes from soils influences the rate of recovery.

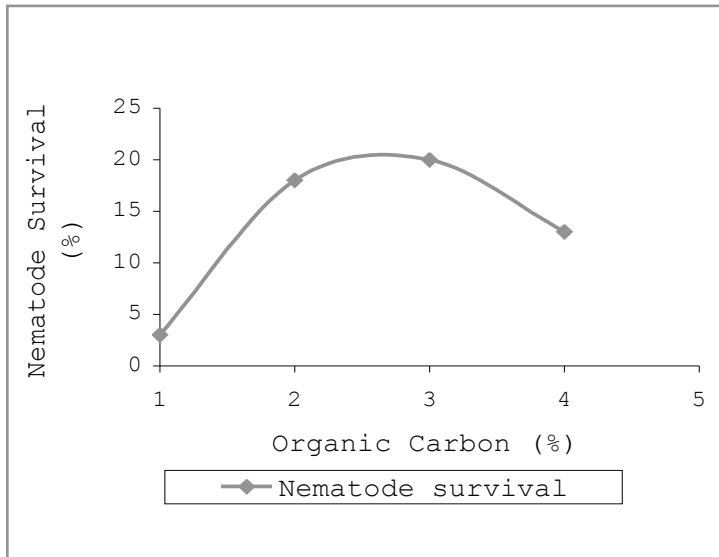
More entomopathogenic nematodes occurred in farmlands than in wild habitats, most likely due to farmers who had loosened soils to improve aeration, and thus, enhanced the survival of nematodes. Small farms with various crops characterized the study area. Different crops have different insect pests, some of which live in the soil, and insect pests may sustain entomopathogenic nematodes in soils. In contrast, wild habitats tend to be composed of one kind of vegetation (forests, pasture, or bushes, for example), which have fewer insect pests, suggesting that the target area for surveys should be determined by the land use. For higher nematode recoveries, farmlands should be targeted, while all habitats should be surveyed

for purposes of discovering the distribution of the nematode fauna. It would be useful to test the effects of tilling land on the virulence of applied nematodes and compare results to those of this study focusing on the survival of natural populations.

Entomopathogenic nematodes survived best in soils of 2-3% carbon (Figure 2). *Heterorhabditis* species preferred soils of greater than 3% organic carbon, while *Steinernema* species were more suited for survival in soils of lower levels of carbon. Carbon improves soil aeration - a prime factor for nematode survival and function, as nematodes cannot survive without oxygen. *Heterorhabditis* species, therefore, seem to be more sensitive to oxygen levels in soils than *Steinernema* species, perhaps one reason why *Steinernema* species are more abundant than *Heterorhabditis* in most surveys.

Soil amendments, such as the addition of manures to increase soil carbon content, may improve survival and effectiveness of *Heterorhabditis* in soils. In general, most entomopathogenic nematodes occurred in soils of pH 5-6. Specifically, however, *Steinernematids* were mainly found in soils of pH less than 6, while *Heterorhabditids* were more suited for survival in soils of pH greater than 6. With the

Figure 2. Effect of soil carbon on entomopathogenic nematode survival.



exception of some *Steinernema* species found in soils with a pH greater than 7, this study showed that extreme scales of the pH were generally unsuitable for nematode survival, implying that low nematode recovery rates are expected from soils of low pH. Furthermore, the test of nematode virulence on the greater wax moth in soils of different levels of pH, for example, showed that virulence increased as pH increased from 4 to 6 for the *Steinernematid* tending to decrease gradually after pH 6, while *Heterorhabditid* virulence tended to increase even beyond pH 7 (Figure 3).

Most nematodes were recovered from 2400-2600 meters above sea level, but the isolates that could reproduce over a longer time were from altitudes less than 2000 meters above sea level. The temperatures and weather of the lower altitudes were similar to those of the culturing laboratory located at 1850 meters above sea level and 18-25 degrees Celsius. Nematodes, therefore, acclimatized quickly to environments similar to those of their origin but not to different environments. Thus, if the purpose of the survey for entomopathogenic nematodes is to recover durable nematode isolates, then the surveys should be targeted at areas with altitudes and weather similar to the laboratory where isolation and culture maintenance will be done.

Soil texture did not significantly affect nematode distribution overall (when all isolates were grouped together); but, specific soil preferences were demonstrated when analysis was done per nematode species. This means that matching nematode species with appropriate soil types would enhance the effectiveness of entomopathogenic nematodes in the biological control of insect pests.

The frequency of entomopathogenic nematodes was higher on coastlines of lakes and constructed dams but was lower along riverbanks and marshlands. Entomopathogenic nematodes of both genera, *Steinernema* and *Heterorhabditis*, were recovered from the same sites near these water bodies, possibly due to the moist conditions and the effects of deposition from higher grounds.

Since nematodes cannot survive without air, marshy soils are unsuitable for nematode survival because they are waterlogged and, thus, poorly aerated. Strangely, however, riverbanks in the study area did not yield nematodes. More surveys targeting riverbanks would establish this rare occurrence as fact or coincidence, as the riverbanks are expected to be moist and also sites of deposition of material from higher grounds. If the aim of the survey, therefore, is

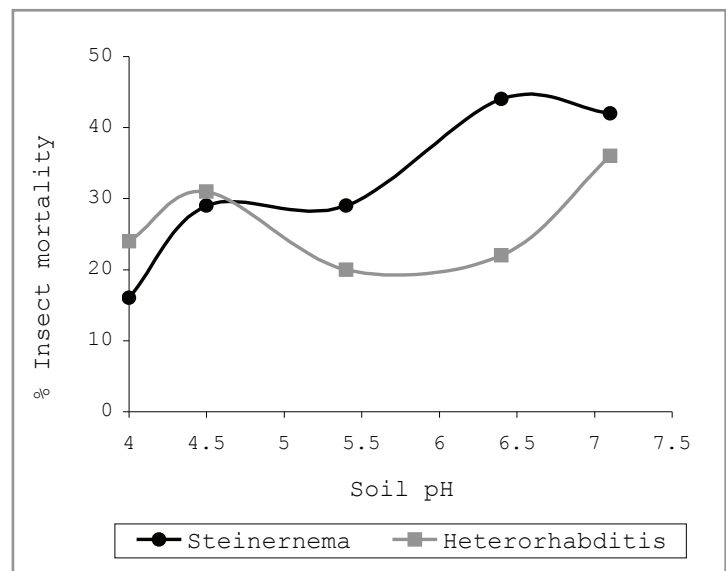
to maximize recovery of entomopathogenic nematodes from the two genera, then the target areas should be those near large water bodies.

The study presented here also found that nematode species that were widely distributed could be reproduced for use for longer time periods than those that occurred less. The wide distribution of any nematode species is a sign of adaptability to wider weather fluctuations and soil conditions.

Practical Implications

Laboratory, greenhouse and field tests are necessary to confirm the efficacy of entomopathogenic nematodes. The results from this study, however, suggest that the usefulness

Figure 3. Effect of soil pH on entomopathogenic nematodes (*Steinernema kariii* and *Heterorhabditis indica*) virulence on insect hosts.



of entomopathogenic nematodes as biological control agents can be predicted from their distribution within a region.

Furthermore, given that more nematodes occurred in farmlands than in wild habitats, farmlands should be targeted to obtain higher nematode recoveries. All habitats, however, should be surveyed for purposes of discovering the distribution of the nematode fauna. It would also be useful to test the effects of tilling land on the virulence of applied nematodes and compare results to those of this study focusing on the survival of natural populations.

The tests to determine the relationship between nematode populations and soil pH suggest that entomopathogenic

nematodes may not be as effective on pests in very acidic soils as they are in soils of near neutral pH. Application of lime to acidic soils may raise the pH, thus enhancing the effectiveness of heterorhabditid nematodes in farms.

Because of the research presented in this brief, the number of nematodes available in the Kenyan entomopathogenic gene bank is now seven; and there is potential for isolating more virulent species in the vast un-tapped regions. It is thus important to conserve the species in a center where they can be retrieved by farmers and scientists, as these nematodes are a naturally occurring alternative to chemical pesticides and a more environmentally-sound and accessible pest control option for smallholder farmers.

Further Reading

Hinga, G., F.N. Muchena, and C.M. Njihia, Eds. 1980. "Physical and chemical methods of soils analysis: A handbook for soil analysis." Nairobi, Kenya: Agricultural Information Center.

Nelson, D. W., and I.E. Sommers. 1982. "Total carbon, organic carbon and organic matter." In *Methods of Soil Analysis, Part 2*, 2nd edition, ed. A.L. Millers and D.R. Keeny. Madison: American Society of Agronomy.

Nguyen, K.B., and C.G. Smart, Jr. 1996. "Identification of entomopathogenic nematodes in the *Steinernematidae* and *Heterorhabditidae* (Nemata: Rhabditida)." *Journal of Nematology* 28(3): 286-300.

Nguyen, K.B., U. Gozel, H.S. Koppenhofer, and B.J. Adams. 2006. "*Heterorhabditis floridensis* n.sp. (Rhabditida: Heterorhabditidae)." *Zootaxa* 1177:1-19.

Thompson, J.D., T.J. Gibson, F. Plewniak, F. Jeanmougin, and D.G. Higgins. 1997. "The CLUSTAL-X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools." *Nucleic Acids Research* 25(24):4876-4882.

Waturu, C.N. 1998. "Entomopathogenic nematodes (*Steinernematidae* and *Heterorhabditidae*) from Kenya." PhD diss., University of Reading, United Kingdom.

About the Author: Shelmith W. Mwaniki is a senior research scientist at the Kenya Agricultural Research Institute (KARI) where she is performing PhD research. Mrs. Mwaniki is registered at the University of Nairobi in the Department of Crop Science and Crop Protection. Email: shelmwani@yahoo.com. Mrs. Mwaniki's U.S. mentor is Dr. John Capinera in the Department of Entomology and Nematology at the University of Florida. Email: capinera@ufl.edu. Dr. Daniel Coyne, a Nematologist at the International Institute for Tropical Agriculture (IITA), is her Consultative Group for International Agricultural Research mentor. Email: D.Coyne@cgiar.org.

With assistance from the Norman E. Borlaug LEAP program, the aim of this project was to trap entomopathogenic nematodes from soils in the selected area and to study the effects of soil factors on their function. The project was also working to develop entomopathogenic nematodes into biological control agents, with emphasis on the sweet potato weevil. The nematodes were identified at the University of Florida with the help of Dr. Khuong Nguyen and Dr. John Canipera, at the International Institute of Tropical Agriculture (IITA Uganda) with the help of Dr. Danny Coyne, and at the Kenya Agricultural Research Institute (KARI-Kabete).

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