

**EFFECT OF PLANT RESIDUES ON SOIL
NEMATODES ACTIVITY AND
THEIR HOSTS**

BY

ENTSAR HELMY TAHA

B.Sc. Agric. Sc. (Entomology), Ain Shams University, 1996

M.Sc. Agric. Sc. (Agricultural Zoology), Ain Shams University, 2002

**A thesis submitted in partial fulfillment
of
the requirements of the degree of**

**DOCTOR OF PHILOSOPHY
in
Agricultural Science
(Agricultural Zoology)**

**Department of Plant Protection
Faculty of Agriculture
Ain Shams University**

2009

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Under the supervision of:

Prof. Dr. Abd El-Samie Hazem Youssef Taha

Prof. Non-Emeritus of Agricultural Zoology, Department of
Plant Protection, Faculty of Agriculture, Ain Shams University
(Principal Supervisor)

Prof. Dr. Shawky Mahmoud Selim

Prof. of Agricultural Microbiology, Department of Agricultural
Microbiology, Faculty of Agriculture, Ain Shams University

Dr. Ahmed Eid Abdel-Megeed Mahgoob

Associate Prof. of Agricultural Zoology, Department of Plant
Protection, Faculty of Agriculture, Ain Shams University

Approval Sheet

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This thesis for Ph.D. degree has been approved by:

Prof. Dr. Ahmed Abd El-Magid Salem

Prof. Non-Emeritus of Agricultural Zoology, Faculty of
Agriculture, Zagazig University

Prof. Dr. Abdalla Shehata Kassab

Prof. Emeritus of Agricultural Zoology, Faculty of Agriculture,
Ain Shams University

Dr. Ahmed Eid Abdel-Megeed Mahgoob

Associate Prof. of Agricultural Zoology, Faculty of Agriculture,
Ain Shams University

Prof. Dr. Abd El-Samie Hazem Youssef Taha

Prof. Non-Emeritus of Agricultural Zoology, Faculty of
Agriculture, Ain Shams University

Date of Examination: 12 / 8 / 2009

ABSTRACT

Entsar Helmy Taha: Effect of Plant Residues on Soil Nematodes Activity and Their Hosts. Unpublished Ph.D. Thesis, Department of Plant Protection, Faculty of Agriculture, Ain Shams University, 2009.

Two strategies, viz., incorporation of organic matter and inundative application of microbes into soil, were followed for controlling root-knot nematode, *Meloidogyne incognita* (Mi), on tomato, *Lycopersicum esculentum* L. cv. Castel Rock, plants. In addition, the impact of these two strategies on the soil-inhabiting entomopathogenic nematode, *Heterorhabditis bacteriophora* (Hb), was also studied. Experiments were conducted under laboratory and screenhouse conditions. Five microbial antagonists, *Bacillus circulans*, *B. megaterium*, *Serratia marcescens*, *Streptomyces* sp., and *Trichoderma viride* were tested *in vitro* against the soil phase of these two nematodes, i.e., the infective second-stage juvenile (J₂) of Mi and the infective third-stage juvenile (IJ₃) of Hb. These microbes were invariably effective against both nematodes, and their effect reduced gradually with the decreasing of their concentrations (cfu/ml). *S. marcescens* was the most effective and *T. viride* was the least. All microbes were more effective against Mi than Hb, e.g., the highest concentration of *S. marcescens* caused 100% and 89% mortality percentages in Mi and Hb, respectively. Also, these microbes reduced the number of invading Mi-J₂s into tomato roots in the screenhouse, causing reduction in nematode galls, egg masses, females and hatched larvae, and improving plant growth. Incorporation of plant materials of different C/N ratios, viz., dried vegetative parts of clover, sunn hemp and marigold, rice straw and sawdust, in soil affected the survival of Hb-IJ₃s in soil under laboratory conditions, causing reduction in penetrating individuals into insect host body. This

reduction increased with the prolonged period of soil phase. In the greenhouse, incorporation of these materials with/without the introduction of the mixture of microbes improved plant growth and reduced nematode infection. However, the effect of plant materials was more pronounced than that of microbes. Prolongation of the decomposition period of plant materials from 45 to 90 days gradually reduced nematode infection and improved plant growth especially in sunn hemp, marigold and clover, in a decreasing order. Number of free-living nematodes increased in amended soil with plant materials.

Key words: Biological control, root-knot nematode, *Meloidogyne incognita*, entomopathogenic nematode, *Heterorhabditis bacteriophora*, microbial antagonists, *Bacillus circulans*, *B. megaterium*, *Serratia marcescens*, *Streptomyces* sp., *Trichoderma viride*, plant organic matter, NPK.

ACKNOWLEDGEMENTS

Foremost and forever, I am gratefully indebted to the Almighty **ALLAH**, most gracious, most merciful.

I would like to express my gratefulness and sincere appreciation to **Prof. Emeritus Dr. Abd-El-Samie Hazem Youssef Taha**, Professor of Agricultural Zoology, Department of Plant Protection, Faculty of Agriculture, Ain Shams University, for suggesting the problem, supervising the work, and valuable suggestions and help in preparing the manuscript.

I would like to express my indebtedness and appreciation to **Prof. Dr. Shawky Mahmoud Selim**, Professor of Agricultural Microbiology, Department of Agricultural Microbiology, and to **Dr. Ahmed Eid Abdel-Megeed Mahgoob**, Associate Professor of Agricultural Zoology, Department of Plant Protection, same Faculty and University, for their continuous help, and for providing facilities throughout the work.

I extend my appreciation and gratitude to **Prof. Emeritus Dr. Abdalla. S. Kassab**, Professor of Agricultural Zoology, Department of Plant Protection, same Faculty and University, for the ready help and contribution he unhesitatingly expressed throughout the study.

Sincere thanks to all the staff members of the Unit of Biofertilizers, same Faculty and University, for their continuous help.

I sincerely thank **Dr Ashraf. B. Abdel-Razik**, Associate Professor of Molecular Genetics, Genetic Department, and **Dr M. M. Elbordiny**, Associate Professor of Soil Science, Soils Department, same Faculty and University, for their help and contributions for statistical and chemical analyses, respectively.

I am gratefully indebted to my beloved mother, my dear husband and our lovely children, Hazem and Rahma, for their unlimited patience and endurance they have burdened throughout the years of my study..

Finally, I have never forgotten my late warmhearted father who was the stimulus of my promotion and success. May the Almighty **Allah**, most gracious, most merciful, bless his soul.

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I. INTRODUCTION

With the intensification of agriculture, losses from root diseases have increased as the buffering effect of biological controls diminished. When biological control is temporarily or permanently inhibited, severe outbreaks of root disease occur. Moreover, the application of pesticides is one agricultural practice likely to affect the natural enemies of nematodes. Fungicides, insecticides and other agricultural chemicals are widely used by farmers throughout the world and it would be naïve to assume that these materials affect only the target organism and have no detrimental effects on organisms such as the nematophagous fungi and the invertebrate predators of nematodes.

Some of man's applications of biological control have proved successful or even spectacular, especially where chemical control has not been economically feasible, and suitable resistant varieties have not been available. Disking into the soil an immature crop (called trap crop) prior to planting host crop prevents the parasite from completing its life cycle. The allelopathic properties of cover crops can also break disease cycles and reduce populations of bacterial, fungal and nematode diseases. Growing a non-host in a rotation with a host crop reduces parasite population.

Currently, nematologists involved in the biological control of nematodes have adopted two main strategies: the manipulation of indigenous natural enemies in soil by using organic matter, and the introduction of selected agents as inundative treatments (**Stirling, 1991**).

The importance of soil organic matter has long been recognized by farmers and the process of incorporating crop residues and animal manures into soil is a practice as old as agriculture itself. The addition of such materials invariably results in an improvement in plant growth. Perhaps the most important effect is that on plant

nutrients. Some organic materials used as soil amendments contain nematotoxic chemicals which are released into the soil and are directly responsible for the suppression of nematode populations. In addition, the action of soil microorganisms on an organic substrate can produce a wide range of chemicals, e.g., ammonia, nitrites, hydrogen sulphide and a wide range of volatile substances and organic acids, can also be detrimental to nematodes.

Some of the reasons for the unfavorable effects of plant residues and organic amendments on plants are quite obvious. Injurious effects may be due to the depletion of nutrients or oxygen, immobilization of nitrogen and phosphorus, or an excess of carbon dioxide, ammonia, or other gases in the soil brought about by microorganisms during the decomposition of the residues. For instance, use of plant residues with high C/N ratios is liable to cause microbial nitrogen immobilization which would affect the nitrogen available to growing plants, especially under conditions of intensive cultivation without sufficiently long fallow periods as exist in Egypt. There is no doubt, however, that the beneficial effects of plant residues far outweigh their detrimental effects.

Because of the intimate relationship between plant-parasitic nematodes and their host, they inhabit the soil in close proximity to the roots rather than the bulk soil mass. The biological control agents must, therefore, be effective at the root-soil surface. When the attributes of plant-parasitic nematodes are considered, it soon becomes apparent that they are not an easy target for biological control agents. They have evolved protective structures and metabolic adaptations which allow them to survive and flourish in a harsh and competitive soil environment. The high reproductive capacity of most plant-parasitic nematodes is one of the features which makes them difficult to control. Once a crop is planted, antagonists need to act quickly, because most the infective second-stage juveniles of the sedentary endoparasitic nematodes penetrate roots within few days.

Entomopathogenic nematodes are soil-inhabiting and extraordinarily lethal to many important soil insect pests, yet are safe for plants and animals. The only insect-parasitic nematodes possessing an optimal balance of biological control attributes are entomopathogenic or insecticidal nematodes in the genera *Steinernema* and *Heterorhabditis* and their symbiotically associated bacteria. The life cycle begins with the only free-living non-feeding developmentally arrested infective third-stage juvenile which occurs in the soil environment where it is vulnerable to natural enemies and soil environmental parameters. Its only function is to seek out and infect new insect host. Therefore, it is faced with a single, vital decision, i.e., whether or not to infect potential host. Since entomopathogenic nematodes suffer antagonism once they are introduced, there will be a need to determine the fate of these nematodes and to understand how to maximize their persistence and mobility (**Gaugler, 1988**). Such studies are likely to provide ecological information of value to plant nematologists because both groups of nematodes have soil-phase and may have some common antagonists.

The two above-mentioned strategies for biological control of plant-parasitic nematodes have been practiced in the present study using plant materials as soil amendment and introducing selected agents as inundative biological control application against root-knot nematode, *Meloidogyne incognita*. It is worthy to explore, in the present study, the effect of plant materials on the soil-dwelling beneficial entomopathogenic nematode *Heterorhabditis bacteriophora*.

II. REVIEW OF LITERATURE

The root system of higher plants is associated not only with an inanimate environment composed of organic and inorganic substances but also with a vast population of metabolically active microorganisms. The plant is markedly affected by the population it has stimulated since the root zone is the site from which mineral nutrients are obtained and through which pathogens must penetrate. Consequently, interactions between the macro- and the microorganisms in this locale can have a considerable significance for crop production and soil fertility. This unique environment under the influence of plant roots is called the rhizosphere.

All organisms in an ecosystem are influenced by abiotic and biotic factors. Nematodes are not exceptions. In an undisturbed ecosystem, many nematode populations might be at equilibrium. When human introduced agriculture into an ecosystem, the equilibrium might be broken and community structure might be changed dramatically so that some nematodes became severe pests of cultivated crops. Nevertheless, these agricultural pests are continuously subjected to attack by a number of natural enemies. The organisms that have adverse effects on nematode populations are collectively called nematode antagonists (**Chen and Dickson, 2004**).

1- Soil nematodes

Two important features emerge from all known details of nematode nutrition (**Nielsen, 1967**):

- (a) the food of nematodes seems, invariably, to be "protoplasm," be it obtained as cell contents, plant sap, the contents of fungal hyphae, algae, bacteria, actinomycetes, protozoa, or other animals;
- (b) the dead organic matter and plant remains of the soil play a considerable role as a substratum for the organisms on which nematodes feed, but do not form part of the nematode diet.

(a) Plant-parasitic nematodes: difficult and diverse target

Plant-parasitic nematodes are usually categorized as soil-inhabiting organisms. All species spend part of their lives in soil with some able to migrate considerable distance and others able to survive for long periods in soil in the absence of the host plant. The microhabitat of nematodes is primarily the labyrinth system of the soil and the water films extending over the soil particles and nematodes. For some species it includes plant roots, either by a superficial association or by actual penetration. Eggs of some species hatch in response to substances that diffuse from roots, all species are attracted to roots, and feeding of ectoparasites and penetration of roots by infective stages of endoparasitic species takes place in the thin layer of soil no more than 2-mm thick surrounding the root. The adult females of some sedentary endoparasites also protrude into this zone, while the eggs of many species are aggregated on the root surface.

The habitat of plant-parasitic nematodes are vitally important when biological control is considered. Essentially, biological control is concerned with interactions between pests and antagonists and it is important to define the battlefield where this 'biological warfare' occurs. Once a crop is planted, plant-parasitic nematodes tend to aggregate near roots and biological control agents must therefore be effective at the root-soil interface (**Stirling, 1991**).

The sedentary endoparasites, root-knot and cyst nematodes are widely distributed and economically important, and most biological control studies have therefore targeted this group of nematodes. However, the endoparasitic habit has proved a major impediment to the development of successful biological control. For most their life cycles, root-knot and cyst nematodes are completely surrounded by root tissue and are therefore protected from soil-borne parasites and predators. Obligate parasites in the genus *Pasteuria* are the only organisms known to consistently infect the endoparasitic stage, but

infection occurs from spores that become associated with nematodes before they invade the root. *P. penetrans* populations parasitic in *Meloidogyne* not only prevent reproduction of the nematode, but also reduce the infectivity of spore-encumbered juveniles (**Stirling, 1984; Davies et al., 1988; Stirling et al., 1990a**).

Plant-parasitic nematodes are not easy target for biological control agents. In addition to the structural features which provide protection against antagonism, the physiological capacity of many plant-parasitic nematodes to survive adverse conditions (**Cooper & Van Gundy, 1971**) may give advantage over some of their parasites and predators. For example, nematodes are the most successful anhydrobiotic animals (**Womersly, 1987**), and are less likely to be affected by dry conditions than many of the organisms that prey on them. However, it is important to recognize that the capacity of nematodes to survive adverse conditions does not give them an advantage over all their antagonists. Some bacterial and fungal parasites, e.g., *Pasteuria penetrans*, *Catenaria auxiliaries*, *Nematophthora gynophila* and *Verticillium chlamydosporium*, have resistant spores which enable them to survive dry periods (**Stirling, 1991**).

The high reproductive capacity of most plant-parasitic nematodes is one of the features which makes them such significant pests, and it also makes them difficult to control. The tremendous capacity for multiplication on a susceptible crop under ideal conditions for the nematode tends to negate the effects of antagonists as high levels of parasitism and predation may do little to diminish final nematode numbers. Since the rate of nematode reproduction is strongly influenced by the host plant, biological control agents are more likely to be successful on crops that are not highly susceptible to nematodes. This has been demonstrated for *Dactylella oviparasitica*, which gives better control of root-knot nematode on peach than on grape (**Stirling et al., 1979**).