Management of plant parasitic nematodes

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ABSTRACT

Diseases caused by plant-parasitic nematodes have significant impact on human life. Every year billions of rupees go down the drain because of the infection caused by these tiny worms. For a long period of time, the damage caused by plant-parasitic nematodes had been attributed to various other pathogens as these tiny worms were not easily noticeable. As a result plant nematology suffered a lot from this stigmatization. Over the years, progresses made in studying the biology of these worms have shed significant light on their secret world.

Plant-parasitic nematodes cause significant losses to agriculture annually. The limited availability and high cost of synthetic nematicides, along with the environmental risks associated with their use, have created a renewed interest in the search for alternative management tactics. One such alternative method for nematode management is the use of plant extracts for their anti-nematode potential. Several natural products of plant origin have been commercialized for management of plant-parasitic nematodes and other soil borne pathogens (Nguyen et al, 2009). Many plant compounds which are active against mammalian parasites have been screened for activity against plant-parasitic nematodes (Oka et al., 2000). Higher plants have yielded a broad spectrum of active compounds, including polythienyls, isothiocyanates, glucosinolates, cyanogenic glycosides, polyacetylenes, alkaloids, lipids, terpenoids, sesquiterpenoids, diterpenoids, quassinoids, steroids, triterpenoids, simple and complex phenolics, and several other classes (Chitwood, 2002). The objective of this research is to evaluate the potential nematicidal activity of water extracts from mustard plant (*Brassica campestris*) Members of the phylum Nematoda (round worms) have been in existence for an estimated one billion years, making them one of the most ancient and diverse types of animals on earth (Wang et al. 1999). They are thought to have evolved from simple animals some 400 million years before the "Cambrian explosion" of invertebrates able to be fossilized (Poinar 1983). The two nematode classes, the Chromadorea and Enoplea, have diverged so long ago, over 550 million years,that it is difficult to accurately know the age of the two lineages of the phylum Nematodes are multi-cellular animals in the group Ecdysozoa, or animals that can shed their cuticle. Also included in this group with nematodes are insects, arachnids and crustaceans. In contrast to some of their relative invertebrates, nematodes are soft-bodied. Thus, very few nematodes have been fossilized (22 species from 11 genera) and exactly what ancestral nematodes looked like remains unknown. While we do not know the morphology of the first nematodes, it is probable that they were microbial feeders in the primordial oceans. The oldest known fossil nematodes are only 120-135 million years old; by then nematodes had diversified to feed on microbes, animals and plants (Poinar et al. 1994, Manum et al. 1994). The oldest fossil nematodes are found in amber and are commonly associated with insects. This is probably due to the fact that tree sap, which fossilizes to make amber, captures and preserves insects and their associated nematodes much more easily than an animal- or a nematode-infested portion of a plant. Much of what we know about the evolution of nematodes is inferred from the comparative anatomy of existing nematodes, trophic habits, and by the comparison of nematode DNA sequences (Thomas et al. 1997, Powers et al. 1993). Based upon molecular phylogenic analyses, it appears that nematodes have evolved their ability to parasitize animals and plants several times during their evolution (Blaxter et al. 1998). One

point is clear; nematodes have evolved to fill almost every conceivable niche on earth that contains some amount of water. Nematodes are extremely abundant and diverse animals; only insects exceed their diversity. Most nematodes are free-living and feed on bacteria, fungi, protozoans and other nematode (40% of the described species); many are parasites of animals (invertebrates and vertebrates (44% of the described species) and plants (15% of the described species). Nematodes were noted early in human history because some serious human diseases are caused by relatively large vertebrate-parasitic nematodes. Some of these nematodes were first described in the ancient Chinese scientific literature as early as 2700 B.C. (Maggenti 1981). Since plant parasitic nematodes often are small and subterranean, there are not many ancient references to phytoparasitic nematodes. One interesting observation suggests that phytoparasitic nematodes were known in antiquity (235 B.C.) because the ancient Chinese symbol for a soybean root-infesting organism resembles in shape an adult female soybean cyst nematode (Noel, 1992). The first described plant parasitic nematodes were discovered in wheat seeds by Needham (1743). Not until the identification of root-knot nematodes on cucumber by Berkeley (1855) and cyst nematodes causing “beet-tired” disease on sugar beets by Schacht (1859), did plant nematology begin to emerge as an important scientific discipline. Nathan A. Cobb, the “father of US nematology,” pioneered agricultural nematology as a USDA scientist in the early 1900’s. The use of soil fumigation to reduce nematode populations and increase crop yields in the 1940’s (Carter) demonstrated that nematodes were significant crop pathogens and ushered in the “chemical era” for nematode management in production agriculture. For a review of the history of plant nematology see the book “General Nematology “by Armand Maggenti (1981); see also the nematode history web sites in Table 1. Today plant parasitic nematodes are recognized as major agricultural pathogens and are known to attack plants and cause crop losses throughout the world. Some estimates suggest they cause 77 billion dollars of damage worldwide each year (Sasser and Freckman 1987). As the full extent of damage caused by plant-parasitic nematodes is recognized by agricultural scientists, the study of the biology of plant-parasitic nematodes will become increasingly important.

MANAGEMNET OF PLANT-PARASITIC NEMATODES

All the control methods are directed at bring down the population of nematodes in soil, low enough to raise the crop profitably. Usually a combination of the control methods is needed to raise crops economically. Nematodes are omnipresent and can be transmitted through a variety of methods. Irrigation water is the prime source of transfer followed by use of infected composts, soil,

Regulatory methods-

1. Quarantine- The principle involved is of exclusion, which implies excluding the possibility of entry of a pest in a region where it is not known at the time of regulatory operation but is likely to establish in the absence of the same.

This is needed as the golden nematode of potato has spread to 40 countries in hundred years. Countries have their vigilance and insist on phyto-sanitary certificate. But in India, domestic quarantine is not carried out. Burrowing nematode has spread from Kerala and Tamil nadu to Maharashtra, Goa, Gujarat, Orissa, Tripura and Lakshadweep Islands too. Some vigilane and cooperative efforts by the farmers and the decision makers are necessary to prevent spread.

Cultural methods-

Crop rotation This is one of the important methods of keeping nematode population under check especially when there are distinct host preferences like the cyst nematodes of wheat, potatoes. Susceptible crops should be grown once in few years and rotating them with non-host crops. Vegetables should not be grown repeatedly in the same field but should be rotated with cereals in order to reduce infestation with root knot nematodes.

Fallowing and ploughing during summer months.- Hot and dry conditions in India are favorable for controlling the nematodes during

the summer months. Nematodes are sensitive to heat and drying action of the sun and the wind. Keeping the land fallow during the summer months and deep ploughing the soil two to three times at intervals of 10 to 15 days is an excellent method to reduce root knot nematodes and others.

Use of organic amendments.- Soil amendments with green manure, compost, oil-cakes [of neem, mahua, mustard, groundnut, cotton, linseed and karanj] sawdust etc. has been found to reduce the nematode populations. These cakes incorporated at the rate of 1 to 1.5 tons/ ha has been found to give good control of root knot nematodes and should be practiced. These encourage the growth of natural enemies of nematodes and the decomposition products of these amendments are also toxic to nematodes.

Use of resistant and tolerant varieties- This is one of the most practical and economical methods of controlling nematodes. Though there are practical difficulties the development of multi-resistant strains of plants to nematodes and fungi is the best choice. Though lot of work has been done on developing varieties of tobacco, cotton, soybean, cowpea, capsicum, tomato, sweet potato much more needs to be done. Also the main drawback sometimes is the development of resistant breaking nematodes which make the process futile.

Physical Methods

Heat treatment of soil - This is a general practice for soil sterilization against all organisms harmful or beneficial. Nurseries use autoclaved soil [30 psi for 30 minutes] for potting. In greenhouses steam is released through perforated pipes running through the soil. Temperatures go up to 82 ^{o} C for 30minutes. Dry heating of soil by burning off the standing crop waste is also practiced in some parts of India.

Hot water treatment of planting material - It is mainly used for transplanted crops, bulbs, rhizomes, root stock and tubers. Effective time and temperatures vary with respect to the plant parts [10 to 60 minutes at 43 to 54 ^{o}C]. Usually dormant bulbs of ornamental plants and root stocks of citrus are given this treatment. Hot water treatment of rice seed at 52 to 55oC for 10 to 15 minutes is a very effective method to control white tip nematode which is transmitted through seed.

Solar drying - Nematode eggs and juveniles are killed if exposed to

Summer ploughing-

Soil solarization or pasteurization-

Oil - Spraying 15% water soluble hydrogenated fish oil also reduces infestation by nematodes. Corn oil, cotton seed oil, ground nut oil and soybean oil reduce the counts of migratory eco-parasitic nematodes.

Floatation of seeds- The control of ear cockle or the 'tundu' disease in wheat is done by removal of nematode galls from seed material and floatation.

Besides these, nematodes are also killed by irradiation, osmotic pressure, ultrasonic and electrical heat. But these methods are uneconomical and not practical.

Chemical Control - This is the most effective method but the main drawbacks are the prohibitive costs and the destruction of normal micro-flora and fauna of the soil. Many of the chemicals are toxic and inflammatory requiring specialized use methods. Many of the chemical nematicides like methyl bromide are now banned in various countries.

Soil fumigants- They may be applied to the soils as liquids, emulsions or granules but the active ingredient spreads in the soil in the form of a gas. Halogenated hydrocarbons called DD, EDB, MBr, and DBCP are commonly used soil fumigants. For successful results, it is essential that the soil be treated is well prepared to a seed bed condition, soil moisture is moderate and uniform and the soil temperatures are between 15 to 30^{o}C at the time of application. Soil fumigants are injected to a depth of 20 to 25cm. Applicators of various types are used from hand injectors to tractor drawn machines which can fumigate 2 to 4 hectares per day. DD and EBD are highly phytotoxic and are applied 3 to 4 weeks before planting. DBCP can however can be used with irrigation water around the roots of plants at recommended doses only.

Use of insecticides which are also nematicidal - many of the organo-phosphorus derivatives and carbamates also possess nematicidal properties. Phorate, , Fensulfothion, Thionazin, Dichlohenthion, Disulfoton, Diazinon,

Fenamiphos Aldicarb, Carbofuran, Methomyl and Vydate have been tested widely all over the world. These are usually applied as granules in furrows. Some of these compounds have not become popular due to high mammalian toxicity and persistence in the soil.

Biological Methods-
Nematodes have their natural enemies in soil like predatory nematodes, fungi, protozoa, viruses, arthropods.
Use of Bio Control agents like fungi, bacteria, nematodes, mites, arthropods etc
[A] Use of nematophagus fungi -Parasites of vermiform [worm like] stages-Fungi that form traps- Several species of soil-dwelling fungi produce traps to ensnare nematodes before they infect them. Traps come in many forms: adhesive hyphae, networks, knobs, rings; and constricting rings. Some trapping fungi can proliferate in soil in the absence of nematodes while others are more dependent on nematodes as a nutrient source for growth.
Arthrobotrys spp.
Monacrosporium spp.
Dactylella spp.
Dactylaria spp.
Geniculifera spp.
Duddingtonia spp
Nematoctonus spp.
Fungi that form adhesive spores (or conidia) - As they move through soil pores, nematodes may encounter these conidia. Some of the fungi are host specific and their conidia will adhere to and infect only a few species of nematode. Although they can be cultured on media, in the soil these fungi are obligate parasites of nematodes and other invertebrates. Examples are -
Hirsutella rhossiliensis
Drechmeria coniospora
Tolypocladium (=*Verticillium*) *balanoides*
Nematoctonus spp.
Fungi that form zoospores- Zoospores are motile spores that are propelled by one or two flagella. When zoospores locate a host, they attach to the nematode cuticle, often near a body opening (mouth, anus, vulva), shed their flagellum, and become sedentary (i.e., encyst). The encysted zoospores infect the nematode cuticle by forming a penetration tube that enters through an orafice or directly penetrates the nematode cuticle.When the resources within the infected nematode are exhausted, the hyphae differentiate to form sporangia. Zoospores are produced within the sporangia and enter the soil via an exit or discharge tube which is formed when the spores are mature. Zoospore-forming fungi are believed to be opportunistic parasites of vermiform nematodes, attacking or colonizing weakened or dead nematodes. A further limitation of zoospore-forming fungi as biological control agents of nematodes is their requirement for wet soils. Zoospore movement is favored in large, water-filled soil pores. Example-*Catenaria anguillulae*
Parasites of Sedentary Stages - There are a large number of fungi that can parasitize nematode eggs, and the sedentary juveniles and females of cyst and root-knot nematodes. These fungi are grouped into two categories: obligate parasites are those that can grow only in nematodes; and facultative parasites are those that can grow to some extent in nematodes as well as on organic matter in the soil.

It will indeed be a wonder if any crop is free from plant parasitic nematodes [PPN]. Many a times in olden days, nematodes have caused people to migrate due to soil sickness. It has been estimated by the International *Meloidogyne* Project that nematodes cause annual looses of 78 billion US dollars in developed countries and more than 100 billion in the developing countries.
Nematode problems are more severe and complicated in warmer than cooler areas, in horticultural than field crops, mono-culture than multi-culture, plantation crops than natural forests and vegetation. Horticultural crops are more efficient producers of biomass and harvestable produce than the agronomical crops. Nematodes pose a constraint to horticultural development and intensive cultivation.
It has been estimated that annually an average 6% loss in field crops, 12% in fruit and nut crops and 11% in vegetables and 10% in ornamental crops is due to nematode infections. Besides causing quantitative losses, nematodes are known to reduce vitamins and minerals in

edible plant parts. Nematode damage is less obvious and many a times goes unnoticed. It causes gradual decline in yield. Nematodes cause complex diseases in association with other soil-borne pathogens.

For a very long period the control of plant parasitic nematode was done using nematicides. These nematicides being chemical in nature has potential to cause destruction to natural habitat. Many countries around the globe banned the use of chemical nematicides for controlling the nematodes, leaving major shortcoming in our ability to limit the yield loss.

Chemical control of plant-parasitic nematodes, essentially, involves the use of synthetic nematicides. However, apart from its very high cost, increased concern for the environment has necessitated a reduction in the amount of nematicides used for nematode control. Additionally, there has been an increase in the intensity of search for other efficient, ecologically sound and safe control methods.

Plant-parasitic nematodes are at their most vulnerable during their active phase in soil when searching for the roots of host plants. Once endoparasitic species have penetrated a root, control with chemicals is more difficult as nematicidal compounds have to be non-phytotoxic and preferably systemic.

A nematicide that can be safely applied to growing plants and is translocated to the roots in sufficiently large amounts to kill endoparasitic or ectoparasitic nematodes has not been discovered. Oxamyl, a systemic compound that is translocated basipetally, is the only commercial product that is used as a foliar treatment, but its use as a liquid formulation is restricted in many countries for toxicological reasons.

There are several nematicides that can be used effectively for nematode pests of annual crops (van Berkum and Hoestra,1979), but there appears to be little prospect for management of nematodes in many susceptible perennial crops without repeated application of nematicides (Tables 1 and 2). Only in certain cases will such treatments be justified economically. Since the discovery and wide-scale use of fumigant nematicides 50 to 60 years ago, a number of products and formulations (Table 1) have been developed for use against several nematode pests, and these are available in most regions of the world (Hague and Gowen, 1987). Only in comparatively recent times have the dangers associated with the manufacture and use of these products become apparent. This has resulted in restrictions on use and sometimes withdrawal from the market. It seems that the age of the traditional fumigants and nematicides has passed, and the opportunity for managing nematodes with synthetic chemicals with broad biocidal activity is declining.

In a quest to develop eco-friendly method to control the damage caused by nematodes, scientists looked in to vast reserve of plants secondary metabolites for controlling the damage. Initial studies done with plant crude extracts has been shown to have significant impact controlling the human and vetinary parasitic nematodes. Success on these model led the scientist to explore the potential of plant extracts in controlling the damage caused by pleant-parasitic nematodes.

Present State of knowledge:-

In the past 20 years three developments have occurred which have had significant effects on the prospects and opportunities for the biological control of plant-parasitic nematodes. First, several nematicides have been withdrawn from the market because of health and environmental problems associated with their production and use (Thomason, 1987). As a result of this, and increasing public concern over the use of pesticides in food production, there has been increased interest in the development of alternative methods of control, including the use of biological agents. Second, it has been demonstrated in several soils that nematophagous fungi and bacteria increase under some perennial crops, and under those grown in monocultures, and so may control some nematode pests, including cyst and root-knot nematodes (Stirling, 1991). Such nematode-suppressive soils have been reported from around the world and include some of the best documented cases of effective biological

control of nematode pests. Finally, a number of commercial products based on nematophagous fungi and bacteria have been developed, but all so far have had only limited success. Their use has been based on empirical research, and it is instructive to consider what might be the key factors for a successful biological control agent for nematodes in order to identify the reasons for the general failure of the products that have been developed.

Biological control is more inconsistent, less effective and slower acting than control normally achieved with chemicals. Although improvements in performance might be expected from more research on individual agents, it seems likely that these limitations are inherent in most biological control agents and that their successful application will depend on integration with other control measures.

The most studied example of natural control of a plant-parasitic nematode concerns the decline of populations of the cereal-cyst nematode, *Heterodera avenae,* under monocultures of susceptible cereals in many soils throughout northern Europe (Kerry, 1982). This is an example of an induced suppression in which the nematode increases to damaging population densities in the second and third cereal crops, but usually declines thereafter to infestations of <5 eggs/g soil, which cause little loss of yield in northern European conditions; it is essential that the nematode is abundant in the early years of the monoculture to support the build-up of the microbial parasites (Kerry, 1988). The decline in nematode populations is mainly caused by two parasitic fungi, *Nematophthora gynophila* and *Verticillium chlamydosporium,* which attack the developing female on the root surface; in suppressive soils 95 to 97 percent of the females and eggs are destroyed (Kerry, Crump and Mullen, 1982). Thus the natural control of cereal-cyst nematode in a range of soils is predictable and effective, but slow acting. Research on the manipulation of natural control has been limited and attention has concentrated on the introduction of specific agents to provide more rapid control that might be commercially exploitable.

It has proved difficult to develop a biological control agent that is effective worldwide for any soil-borne disease. Despite much research effort only two agents have had widespread success: *Phlebia gigantea* for the control of *Heterobasidion annosum,* which spreads from tree stumps to the roots of adjacent trees, *and Agrobacterium radiobacter* that is applied as a root dip to transplants for control of crown gall caused by *A. tumefasciens.* For both, the agents are applied in high concentrations as inundative treatments to a readily accessible site of action (the cut surface of a tree stump or the bare roots of a transplant) and protection from the diseases for a relatively short period provides long-term control (Deacon, 1991). In most situations where nematode control is required, there is a need to provide long-term protection to a relatively inaccessi- ble and growing root system without the use of inundative treatments that are likely to be impractical and uneconomic. Hence the development of biological control agents for plant-parasitic nematodes is likely to be difficult and to require a detailed understanding of the biology and ecology of the agent and the nematode target.

The farming system in which biological control is applied has a marked effect on the way the agent is used (Davies, de Leij and Kerry, 1991). In general, growers in developed agriculture require an agent that can be applied to crops grown in monocultures over large areas using standard application machinery, so a formulated product with a good shelf-life that can be applied at low dosages is required; seed treatments are preferred for most arable crops. Little research has been done on the mass production and formulation of biological control agents for nematodes. Some organisms, such as rhizosphere bacteria, can be applied as seed treatments (Oostendorp and Sikora, 1989), but such applications tend to provide short-term control and are only useful in reducing the invasion of roots by nematodes that have a single generation in the growing season. In subsistence farming systems, crops tend to be grown in mixed stands in relatively small areas (often less than one hectare) and labour inputs are often large. As a consequence, relatively

large application rates (up to one tonne per hectare) of an unformulated agent could be mixed into the soil by hand, as long as the organism could be produced cheaply and locally. Thus, the initial exploitation of biological control agents for nematodes may be in developing countries (Hussey, 1990). However, if agents are only effective against specific nematode pests, and their efficacy is dependent on pest densities, then their effective use will require expert advice that may not be available in many developing countries.

Preliminary work done on the lines

Too often, biological control agents have failed because they have been used before a basic knowledge of their ecology and biology has been established. The importance of such knowledge can be seen from work at Rothamsted aimed at the development of *V. chlamydosporium* as a biological control agent for root-knot nematodes. Stirling (1991) describes the design and methods used in the conduct of biological control experiments, which are more complex than those required to assess the efficacy of a nematicide. He identified five key aspects in setting up an experiment to evaluate a biological control agent and these are presented below in a slightly modified form.

· The test organism and any organic amendment should be applied at practical application rates; 0.1 percent w/w soil is equivalent to 2.5 tonnes/ha and should represent a maximum dose. Tests should always be performed in a non-sterilized soil with a natural residual soil microflora.

· Appropriate treatments as well as an untreated control should be included if the organism is added with a substrate. These treatments should include the substrate alone, the organism alone, and the autoclaved colonized substrate. Too often, untreated controls are compared only with large applications of the organism and substrate and this does not allow separation of the effects of the agent from the effects of the substrate. In several tests reported in the literature, application of the substrate alone has decreased nematode populations to the same extent as the substrate colonized by the agent, and there is no clear evidence of biological control.

· Population densities of the agent under test should be monitored to ensure that it has survived in soil throughout the period that activity against the nematode target is required. Such monitoring may require the development of selective media, which can be a difficult and time-consuming task.

· Nematode mortality caused by the organism under test should be measured to assess whether differences between nematode population densities in treated and untreated soil relate to the levels of kill caused by the agent. Infection levels are relatively straightforward to estimate for most parasites, but repeated sampling is required to determine total kills. The effects of agents which produce toxins or have indirect effects on nematodes through competition, the modification of root exudates or the colonization of feeding cells, can only be measured by assessing their impact on nematode development.

· The impact of the soil environment, host plant and nematode should be tested as these are likely to affect the efficacy of the biological control agent, and could account for the lack of activity of potential agents in specific test conditions.

In a review of the literature less than 15 percent of experiments purporting to demonstrate biological control caused by *Paecilomyces lilacinus* satisfied the above criteria (Kerry, 1990). Although this is an unsatisfactory situation that must be remedied, the difficulties in conducting carefully controlled and monitored experiments should not be underestimated.

Isolates of *V. chlamydosporium* differ markedly in their growth and sporulation *in vitro* (Irving and Kerry, 1986), and in their virulence, saprophytic competitiveness and rhizosphere competence (Kerry and de Leij, 1992). Such differences between isolates of the same species of micro-organism are common and there is a need for simple laboratory-based screening methods to select the most promising isolates for further testing. *Verticillium chlamydosporium* isolates were collected from infested nematode females and eggs in suppressive soils around the world. Hence, they were isolated from the niche in which they

would be required to be active as a biological control agent and from soils most likely to yield active isolates. Once isolated in pure culture using standard techniques, the *in vitro* growth requirements were determined.

Virulent isolates were selected by counting the number of nematode eggs parasitized after exposure to the fungus on agar in a standard test (Irving and Kerry, 1986). Tests for proliferation in the rhizosphere (de Leij and Kerry, 1991) and growth in soil (Kerry, 1991) were also used to select promising isolates. Although simple laboratory-based screens help eliminate many isolates that show insufficient activity to justify further testing, selected isolates will not necessarily be active in the field. As it may take 10 to 16 weeks to investigate adequately the performance of different isolates against cyst and root-knot nematodes in pot tests, relatively few can be screened.

The method of mass culturing of *V. chlamydosporium* for experiments can have a marked effect on the subsequent survival and proliferation of the fungus in soil. Inoculum produced in shaken liquid cultures consists mostly of hyphae and conidia, which require an energy source to ensure proliferation in soil (Kerry, 1987), whereas on solid media large numbers of chlamydospores are produced and these can be added to soil in aqueous suspension and rapidly establish the fungus (de Leij and Kerry, 1991).

The development of a semi-selective medium (de Leij and Kerry, 1991) has enabled detailed studies to be made on changes in relative abundance of the fungus in soil and on roots. Some isolates of *V. chlamydosporium* may be extremely abundant in soil but unless they are capable of colonizing the rhizosphere they do not parasitize the eggs of root-knot nematodes. Growth in the rhizosphere differs markedly between plant species, e.g. tomato, cabbage and maize roots support much growth, whereas sorghum, pepper and cotton are poor hosts (Table 4). Colonization by the fungus is confined to the rhizosphere and rhizoplane and there is no spread into root tissue; no lesions have been observed on roots grown in soil treated with *V. chlamydosporium* and there have been no detrimental effects on the growth of a range of crop species. A tenfold reduction (from 10^4 to 10^3 chlamydospores/g soil) in the amount of fungus applied to soil had no effect on the extent of colonization in the rhizosphere (de Leij, Davies and Kerry, 1992); the ability to proliferate on the root surface where the fungus is required to control nematodes is an important characteristic which may allow significant reductions in the amount of inoculum applied to soil.

The efficacy of *V. chlamydosporium* as a biological control agent for root-knot nematodes is affected by three key factors: the amount of fungus in the rhizosphere (Table 4); the rate of development of eggs in the egg masses; and the size of the galls in which the female nematodes develop. In large galls female root-knot nematodes may produce egg masses which remain within the gall and are not exposed to parasitism by *V. chlamydosporium,* which is confined to the rhizosphere. Hence, *V. chlamydosporium* is less effective in controlling root-knot nematodes in heavily infested soils and on highly susceptible crops because large galls are formed on the roots and many eggs escape parasitism. *Verticillium chlamydosporium is* unlikely to be useful in these situations where a grower would normally apply a nematicide. Also, at temperatures above 25°C eggs may complete their embryonic development and hatch before the fungus has completely colonized the egg mass; at 30°C about 30 percent of eggs of three root-knot species hatched and the second-stage juveniles escaped from the egg mass before the eggs were killed (de Leij, Dennehy and Kerry, 1992). These studies in pot tests, if supported by field experiments, help to define the conditions in which *V. chlamydosporium* might be used successfully for control of root-knot nematodes. It is only from such detailed studies that the limitations and requirements of the fungus can be assessed.

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