Sonderheft 297
Special Issue

Landbauforschung
Völkenrode
FAL Agricultural Research

Investigations on possibilities to improve the antiphytopathogenic potential of soils against the cyst nematode *Heterodera schachtii* and the citrus nematode *Tylenchulus semipenetrans*

Hazem Abdelnabby

Bibliographic information published by Die Deutsche Bibliothek
Die Deutsche Bibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data is available in the Internet at http://dnb.ddb.de .

2006

Landbauforschung Völkenrode - FAL Agricultural Research
Bundesforschungsanstalt für Landwirtschaft (FAL)
Bundesallee 50, 38116 Braunschweig, Germany

landbauforschung@fal.de

Preis / Price: 8 €

ISSN 0376-0723
ISBN-10: 3-86576-021-X
ISBN-13: 978-3-86576-021-0

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

Conc.	Concentration
C_t	Total Carbon
DS	Developmental Stages
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen
EM	Egg mass
ES	Experimental Soil
FAA	Formalin Acetic Acid Alcohol
FAO	Food Agricultural Organisation of the United Nation
Fig.	Figure
Filtr.	Filtrate
ft	feet
g	gram
GFM	Gliotoxin Fermentation Medium
h	hour
Imm. stage	Immature stage
ind.	individual
Inoc.	Inoculated
IPM	Integrated Pest Management
IU	International Unit
J_2	Second Stage Juvenile
K_{CAL}	Potassium by Calcium-Acetate-Lactate (CAL)-extraction
NOCV	Navel Orange on *Citrus volkameriana*
NOPT	Navel Orange on *Poncirus trifoliata*
OMF	Optimum mineral fertilisation containing essential nutrients
N_t	Total Nitrogen
OSS	Organic Soil Substance
P_{CAL}	Phosphorus by CAL-extraction
PDA	Potato Dextrose Agar
Pf	Final population
Pi	Initial Population
P.R.I.	Population Reproduction Index
PTB	Physikalisch-Technische Bundesanstalt
PV	Prominence Value
rpm	round per minute
Tab.	Table
Treat.	Treatment
USDA	United States Department of Agriculture
WC	Water Content
WHC_{max}	Maximum Water Holding Capacity
wt.	Weight

LIST OF TABLES

LIST OF FIGURES

1 Introduction

1.1 Background

Soil is a living system that represents a finite resource vital to life on earth. It is a complex mixture of minerals, water and air, organic compounds, and living organisms. Soils are the central organiser of the terrestrial ecosystem. Their colloidal and particulate constituents, including minerals, organic matter, and microorganisms, are not separate entities; rather, they constantly interact with each other (Huanga et al., 2005).

Agroecosystems are man-made. Several events, such as soil tillage, fertilisation, and application of pesticides during a cropping season, as well as the export of harvest products, interfere severely with the soil-borne food webs by cutting the functional chains (Larink, 1997). Soil quality is one of the significant agroecosystem components for which management efforts must intensify in order to achieve sustainability. This is defined as 'capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation' (Karlen et al., 2003). The soil quality is estimated by soil physical, chemical and biological factors (Karlen et al., 2001). Soil health, however, focuses more on the biotic components of a soil, e.g., the maintenance of soil organisms and their proper functioning as regulators of nutrient cycling and therefore of soil fertility. According to the recent definition by Doran and Safley (1997), the soil health is 'the continued capacity of soil to function as a vital living system, within ecosystem and land use boundaries, to sustain biological productivity, promote the quality of air and water environments and maintain plant, animal and human health'. Various biological indicators are related to soil quality, for example microbial biomass, soil respiration, soil enzyme activities, earthworm number and nematode number (Liebig and Doran, 1999; Andrews and Carroll, 2001; Karlen et al., 2001; Andrews et al., 2003).

The use of soil fauna as indicator offers different possibilities. Single species bioassays are important to assess the effects of single stressors and bioconcentration studies. However, these tests are often realised in laboratory experiments, with soil samples transferred in experimental systems and spiked with contaminants. Experiments on community level are ecologically more relevant. They integrate the interactions of all soil factors including management and pollutants effects (Schloter et al., 2003). Among the groups of soil fauna, nematodes are ideally suited to use as indicators of soil quality (Bongers, 1990). Different tests in ecotoxicology, realised for single species as well as for

communities, showed the suitability of nematodes as bioindicators of soil quality (Traunspruger and Drews, 1996; Freeman et al., 2000; Peredney and Williams, 2000; Haitzer et al., 1999). Furthermore, the environmental disturbance can be measured ecologically using the Maturity Index (MI) based upon relative selection of fast colonisers (c) versus slow persisters (p) growing forms of nematodes in different environments (de Goede and Bongers, 1994).

The food web stability (Fig. 1.1) in the conventional farming system is challenged by the yearly ploughing the soil, which reduces the numbers of organisms that displace or prey on plant-parasitic nematodes, while bringing more nematodes to the surface from deeper soil. If the same host crop is planted year after year, plant-parasitic nematodes may increase to damaging levels. The root-feeding nematodes are very opportunistic, and are among the first organisms to invade after a disturbance (Dropkin, 1980; Ingham, 1996). Keeping these facts in mind, it is important to actively manage soil biology using minimum-tillage practices, compost, animal manures, green manures, cover crops, and crop rotations. These practices help promote the growth of beneficial organisms while suppressing plant parasites (Barker and Koenning, 1998). Organic soil amendments are now widely recognised as a ‘non-conventional' nematode management option (Muller and Gooch, 1982; Akhtar and Mahmood, 1996). Soil pH, nitrogen form, and the availability of nutrients can play major roles in disease management. Adequate crop nutrition makes plants more tolerant of or resistant to disease. Also, the nutrient status of the soil and the use of fertilisers and amendments can have significant impacts on the pathogen environment (Sullivan, 2004). Management practices that add nutrients to the soil enhance soil biological activity. However, any positive effects of synthetic fertilisers occur indirectly, through the stimulation of plant growth. Application of fertilisers such as ammonium nitrate, urea, or super-phosphate demonstrated toxic effects on soil fauna. Legumes, which act as a source of organic matter and nitrogen support, cause larger numbers and greater activity of soil microbes than inorganic nitrogen sources (Liebhardt, 1991).

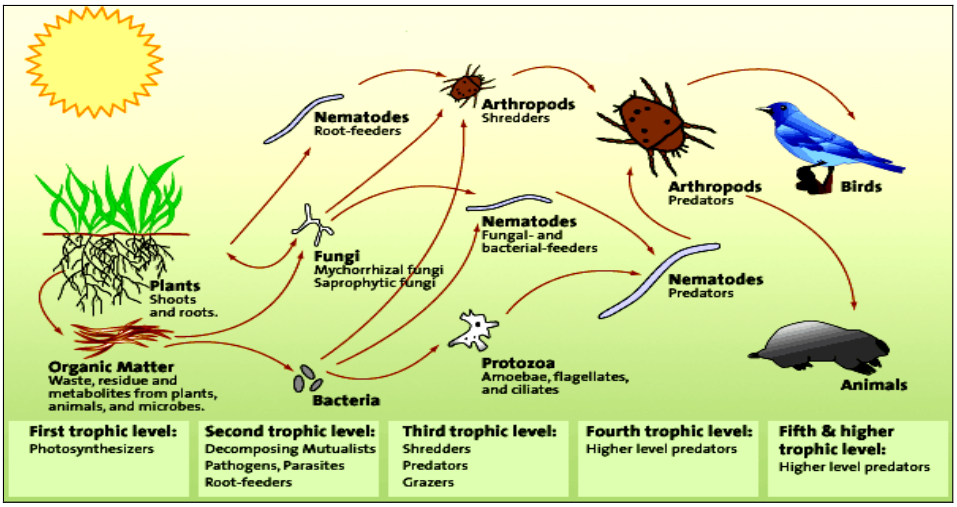


Fig. 1.1: The soil food web http://www.totallyorganic.net/how-it-works.html

Interest in biological control has increased recently, fuelled by public concern over the use of chemicals in the environment in general and the need to find alternatives to the use of chemicals for disease control. However, microorganisms as biological control agents typically have a relatively narrow spectrum of activity compared with synthetic pesticides and often exhibit inconsistent performance in practical agriculture, resulting in limited commercial use of biocontrol approaches for suppression of plant pathogens (Backman et al., 1997). Toxins produced by microorganisms are typically secondary metabolites featuring peptides, polypeptides and non-peptide antibiotics (Gilquin et al., 2003). The search for new microbial strains to use as a source of biological nematicides is an important goal for those seeking to reduce the significant economic damage caused by plant-parasitic nematodes. Fungi exhibit a range of specificities and mode of action in their antagonistic activity toward nematodes, offering an extensive pool of potential candidates to test (Siddiqui and Mahmood, 1996). Like other microbes, fungi can directly parasitize nematodes or secrete nematicidal metabolites and enzymes that affect nematode viability (Nitao et al., 1999). Mycotoxin production and toxic effects vary according to the fungal strain, culture medium and target organism (Cayrol et al., 1989).

There is still a need for alternative, environmentally friendly measures or compounds for effective nematode control to be developed. One way of searching for such nematicidal compounds is to screen naturally occurring compounds in plants. Plants are an important source of naturally occurring pesticides. Many compounds with nematicidal activity have been found in plants, including alkaloids, diterpenes, fatty acids, glucosinolates, isothiocyanates, phenols, polyacetylenes, sesquiterpenes and thienyls

(Chitwood, 2002). Some plants contain compounds that affect the behaviour of other organisms in the plant environment. For example, Sudan grass and sorghum contain a chemical, dhurrin that degrades in the course of tissues injuries into hydrogen cyanide, which is a powerful nematicide (Luna, 1993; Forge et al., 1995; Wider and Abawi, 2000). Several groups of researchers are attempting to develop phytochemical based strategies for nematode control. Compounds involved in plant-nematode interaction include repellents, attractants, hatching stimulants or inhibitors, and nematotoxins (Stirling, 1991a). Essential oils of some plants and/or their components have been tested for nematicidal activity in vitro and in soil (Chatterjee et al., 1982; Soler-Serratosa et al., 1996; Oka et al., 2000). Several benefits may result from the identification of the specific phytochemicals (Fig. 1.2) involved in these interactions (Chitwood, 2002).

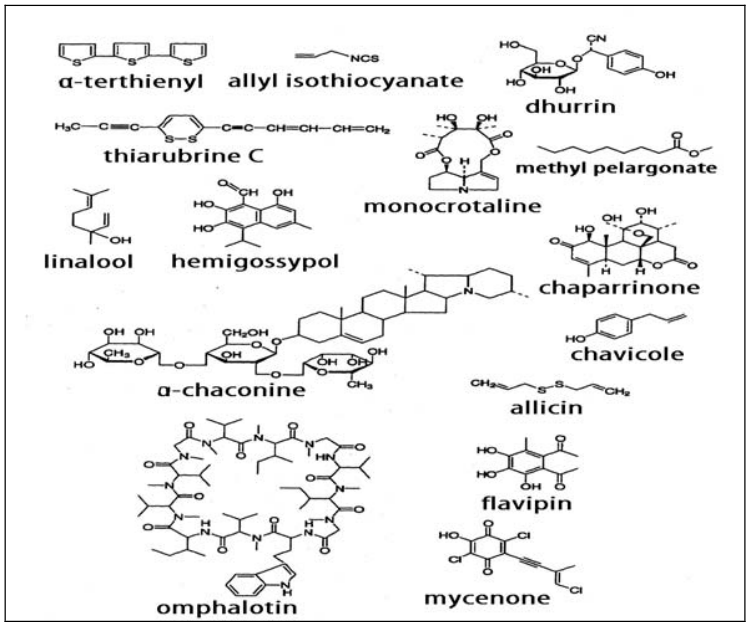


Fig. 1.2: Chemical structure of the most popular phytochemicals with nematotoxical activity (from Chitwood, 2002)

We need to have a sound knowledge of the population dynamics of nematodes, of the threshold levels needed to cause economic damage, of the role of plant nutrition and its relation to plant resistance, of the role that parasites, predators and other organisms in soil play in regulating nematode population, and the complex interrelationships that occur between nematodes and other components of the soil ecosystem.

The problem addressed by this research is the enhancement of soil antiphytopathogenic potential against the most economically important plant attacking nematodes, the sugar beet cyst nematode *Heterodera schachtii* in Germany and the citrus

nematode *Tylenchulus semipenetrans* in Egypt, using safe methods with emphasis on improvement of soil health and quality.

1.2 Objectives

Understanding integrated nematode management will help nematologists curtail the nematode exacerbated damage so as to pave the way for excellent yield and desirable qualities. Therefore, the present research was mainly outlined to study the following objectives:

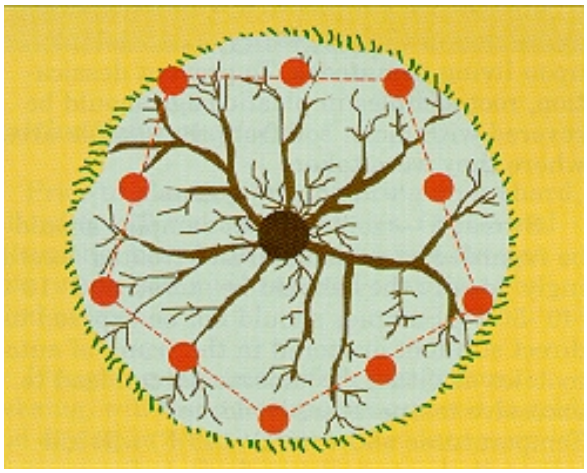
1. Monitoring soil quality against plant parasitic nematodes depending on the cyst nematode *Heterodera schachtii* and the citrus nematode *Tylenchulus semipenetrans* as indicators using:
    - *i.* Organic and inorganic fertilisers
    - *ii.* Biological control
    - *iii.* Plant extracts
2. Evaluation of citrus rootstocks as a citrus nematode control strategy.
3. Identification of the nematode seasonal population peaks to determine the most suitable time for applying nematode control strategies.

2 Materials and methods

2.1 Sampling procedures

Soil samples from the citrus experimental orchard of the Faculty of Agriculture (Moshtohor), Zagazig University, Egypt (30˚ 21´ N, 31˚ 13´ E, 46 ft above sea level) were collected using a "T" type sampling tube, trowel, or shovel.

Samples were obtained from the area of feeder roots by digging the soil at a depth of 15-30 cm after removing the top layer of 5 cm (Fig. 2.1). Each sample was composed of three sub-samples.



http://www.omafra.gov.on.ca/english/crops/facts/nematode.htm

Fig. 2.1: Sampling strategy (from feeder roots around the citrus trees)

Samples were transferred to the laboratory in labelled polyethylene bags and processed soon after collection or stored at 4°C until extraction. Afterwards, numbers of free second stage juveniles were estimated.

2.2 Biological methods

2.2.1 Nematode propagation in soil

Cysts of the sugar beet cyst nematode *Heterodera schachtii* were extracted from the experimental field of the Institute of Plant Nutrition and Soil Science, Federal Agricultural Research Centre, Braunschweig, Germany (10˚ 27´ E, 52˚ 18´ N, 81 m above sea level). The soil is composed of 46 % sand, 47 % silt and 7 % clay, hence, it is characterised as strong silty sand following the German classification system (AG Boden, 1994) or sandy loam/loam according to FAO or USDA classification. Cyst nematode eggs at 3000 eggs

100 g^{-1} soil were introduced into sterilised soil planted with 10 cm high sugar-beet *c.v.* Tatjana as inoculum source and placed in a greenhouse at 25-30 °C.

Citrus nematode, *Tylenchulus semipenetrans* juveniles were originally extracted from the infected citrus orchards of the experimental farm of the Faculty of Agriculture at Moshtohor, Zagazig University, Egypt (30˚ 21′ N, 31˚ 13′ E, 46 ft above sea level) and propagated on sour orange seedlings grown in 50 cm pots filled with sandy loam soil.

2.2.2 Inoculation procedures

In greenhouse experiments, the inoculation was achieved by pouring the eggs or nematode water suspension into 4 holes (3-5 cm depth) around the root system, which were immediately covered and mixed with soil.

In the case of cyst nematode experiments, each pot was inoculated with 3000 free 2^{nd} stage juveniles, while the inoculation per pot was 5000 eggs in citrus nematode experiments.

2.2.3 Nematode extraction, counting and identification

At the end of the experiments, the soil of each pot was removed and mixed carefully. Aliquots of 250 g soil were processed for nematode extraction and recovering according to Christie and Perry (1951). The samples were transferred to the laboratory in labelled polyethylene bags. Each sample was wet-sieved through 100 and 400 mesh sieves. Active nematodes were separated from debris and fine soil particles using a Baermann pan fitted with a soft tissue paper. After 48 hours, the water suspension containing migrated nematodes was collected and concentrated to 50 ml in a glass vial using a 400 mesh sieve.

After agitating, 1 ml of nematode suspension was administered into the counting chamber. The number of second stage juveniles was estimated. The roots were washed carefully in tap water. The number of adult females was recorded. Afterwards, they were killed and fixed in formalin acetic acid alcohol (FAA, 1:1:18 vv). The number of eggs inside cysts and egg masses and pre-adult stages per g roots was estimated. The obtained data were normalised to the whole root fresh weight.

Cysts and females of sugar beet cyst nematodes were extracted from soil by suspending the soil in water and obtaining the cysts through pouring. For this purpose, soil samples were washed through the 840 µ aperture (20-mesh) sieve. Cysts were rinsed thoroughly and collected. An aspirator was used to remove cysts from wet debris (Shepherd, 1987).

The recovered nematode genera and species were identified according to Williams (1960), Sturhan (1966), Loof (1978), Sasser and Carter (1985), Siddiqi (1986), Jepson (1987) and Sharma (1998).

2.3 Vegetative measurements

At the end of the experimental period, the soil in pots was well irrigated before removing the plants. Roots were washed in a gentle flow of water. Plant height [in cm] from the soil surface to the highest growing top of the plant was measured as well as the length of the root system [in cm] from the soil surface to the root tips. Shoots and roots were separated, weighed and dried in an oven at 70 °C for three days. Fresh and dry weights of shoots and roots were recorded separately.

2.4 Nematode control strategies

2.4.1 Organic and inorganic fertilisers

As organic material, commercial potting mixture (Floragard®) (N 170, P 170, K 240 mg L^{-1} and 70 % water content) was mixed with soil at concentrations of 3 % (55 g pot^{-1}), 12 % (199 g pot^{-1}) and 40 % (439 g pot^{-1}). Total weight of each pot was 1500 g.

Pigeon excrements were obtained from pigeon farms in Menofeya governorate, Egypt. The excrements were air-dried, ground to pass through a 2 mm sieve, and stored in plastic bottles until mixture with soil at doses of 25, 50 and 100 g pot^{-1}. Crushed garlic was prepared using fresh garlic cloves of *Allium sativum* L. that was mixed with soil at doses of 25, 50 and 100 g pot^{-1}. The total weight of each pot was 2500 g.

As optimum mineral fertilisation (OMF), the commercial fertiliser Fischer Gartenland® Super Flüssigdünger (N: P_2O_5: K_2O = 6:6:6 + micronutrients B, Cu, Fe, Mn, Mo and Zn) was applied as soil drench fertiliser at concentrations of 1.75, 3.5 and 7 ml L^{-1} twice weekly at a rate of 50 ml pot^{-1}. Ammonium nitrate NH_4NO_3 (N =33.5 %) was applied twice at rates of 5, 10 and 20 g pot^{-1}, the first time at the beginning of the experiment and the second one month later.

2.4.2 Organic and amino acids

Ascorbic and salicylic acids were used at 1, 2 and 4 g L^{-1} as soil drench, while the commercial mixture of amino acids, Amino green® II (amino acids 15 %, Fe 2.9 %, Zn 1.4 %, Mn 0.7 %) produced by Elnasr for fertilisers and biocides was used as foliar application at rates 0.5, 1.0 and 2.0 ml L^{-1}. Applications of the organic and amino acids - even as foliar

spray or soil drench - were carried out twice, at the beginning of the experiment and one month later.

2.4.3 Microbial agents

Six different antagonistic fungi isolates and one species of bacteria were tested. Four antagonistic fungi isolates, *Arthrobotrys oligospora*, *Fusarium equiseti*, *Trichoderma harzianum* and *Verticillium lecanii,* were obtained from the German National Resource Centre for Biological Material (DSMZ), Braunschweig, Germany. Two antagonistic fungi, *Dactylella brochopaga* and *Nematoctonus concurrence,* were obtained from the Agricultural Research Centre, Plant Disease Department, Cairo, Egypt. The bacteria *Bacillus thuringiensis* was applied in the form of the commercial bacterial biocide Agerin® (32000 IU mg^{-1}) produced by Biogro International-Egypt.

The antagonistic fungi were grown separately on potato dextrose agar medium (PDA) for 9 days at 25 °C. Afterward, they were grown using Gliotoxin Fermentation Liquid Medium (GFM) (Brian and Hemming, 1945) which is composed of 25 g Dextrose, 2 g Ammonium tartrate, 2 g KH_2PO_4, 1 g $MgSO_4$, and 0.01 g $FeSO_4$ in a total volume of 1000 ml distilled water.

Culture filtrate preparation

The investigated fungi species were grown using the media GFM in 500 ml conical flasks. These flasks were incubated at 25 °C under complete darkness conditions (Abdel-Moity and Shatla, 1981). After 11 days, the culture was blended for 3 minutes and the mixture was filtered first by filter paper, afterwards the filtrate was centrifuged for 15 minutes at 3000 rpm to separate the fungal spores. Filtrates were sterilised using centre glass (G4). Three different concentrations (100, 75 and 50 %) of sterilised fungal filtrates were applied. The filtrates were added to the plant roots at a rate of 10 ml $plant^{-1}$. The commercial bacterial biocide Agerin® (*Bacillus thuringiensis*) was applied at rates of 1, 2 and 4 g pot^{-1}.

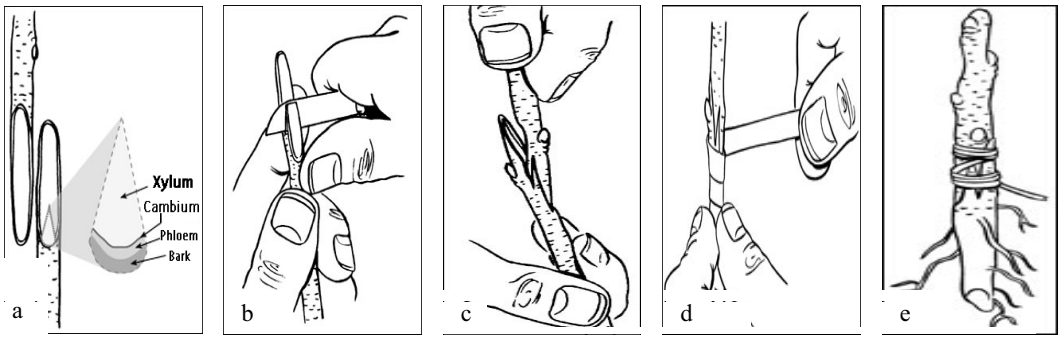
Applications of the microbial agents - even as fungi or bacteria - were carried out twice, at the beginning of the experiment and one month later.

2.4.4 Navel orange on resistant rootstocks

Navel orange scions on the resistant rootstocks Japanese Bitter Orange (*Poncirus trifoliata* (L.) Raf.) NOPT and `Volkamer' lemon (*C. volkameriana* Tanaka) NOCV were

used against the citrus nematode *Tylenchulus semipenetrans* and compared to the control (navel orange on sour orange).

The "whip graft" technique (Fig. 2.2) was chosen for the reselected rootstocks to allow the grafted rootstocks to develop more rapidly. Small branches (not more than ½-inch in diameter) were chosen. Straight and smooth, slanting cut about 1½ inch long on both the scion and the stock was made (a). A "tongue" was cut on both scion and rootstock by slicing downward into the wood (b). The two parts were fitted tightly together (c). The union was carefully covered with grafting compound and bound with budding rubber (d). Finally, whip and tongue graft with scion attached to the root system (e).



http://muextension.missouri.edu/xplor/agguides/hort/g06971.htm

Fig. 2.2: Processes of the whip graft technique

2.4.5 Plant extracts

Thuja orientalis Linnaeus 1753 which is also called *Biota orientalis* (Linnaeus) Endlicher; *Platycladus stricta* Spach; *Thuja chengii* Bordères and Gaussen; *T. orientalis* var. *argyi* Lemée and H. Léveillé (Fu et al., 1999) is a native Chinese tree and belongs to the Cupressaceae family with a maximal height of more than 20 m (Fig. 2.3). It has coniferous pyramidal features, with flattened branches and twigs in one plane, bearing small scale-like leaves. All the year the leaves are green, with the lower side showing a brighter green where resin glands also reside. Small, 1–2 cm long, green to brown coniferous pins contain the seeds (British Herbal Pharmacopoeia, 1983).

www.giftpflanzen.com

Fig. 2.3: Tree of life (*Thuja orientalis*)

Thevetia neriifolia Juss. ex Steud. which is also called *Cascabela thevetia* (L.) Lippold, *Cerbera peruviana* Pers., *Cerbera thevetia* L. or *Thevetia peruviana* (Pers.) K. Schum belongs to the order Apocynales and Apocynaceae family and grows to 15 feet with long, narrow dark green leaves (Fig 2.4). It is a native of tropical America; especially Mexico and the West Indies, but has naturalised in tropical regions worldwide. It is also known under the names of Bestill tree, Peruvian yellow oleander, Yellow oleander (English) and Thevetie (German) (Wang, 1996).

www.enchantedgardenaz.com

Fig. 2.4: The yellow oleander (*Thevetia neriifolia*)

The experimental plants were obtained from the ornamental gardens of the Faculty of Agriculture at Moshtohor, Zagazig University, Egypt.

Fruits of *Thuja orientalis* (Cupressaceae) and fresh leaves of *Thevetia neriifolia* (Apocynaceae) were collected and an extract of each was prepared in two solvents of

increasing polarity, hexane and ethyl acetate, respectively. Fresh leaves and fruits were thoroughly washed in sterile water. The water on the surface area was allowed to dry by spreading the leaves and fruits on a clean bench at room temperature. The dry plant material was ground in an electric mill to fine powder. Each gram of the plant tissue was soaked in five ml of each solvent for 3 days with shaking. Afterward, the solution was filtered through filter paper. The solvent was evaporated under reduced pressure using a rotary evaporator. The temperature of the water bath was 50 °C. Each crude extract was weighed and kept in a refrigerator at 4 °C until preparation of four dilution levels of 10, 5, 2.5 and 1.25 % (w/v) using distilled water and application as soil treatment. Applications of the plant extracts were carried out twice, in the beginning of the experiment and one month later.

2.4.6 Carbofuran

Inoculated plants were treated by the systemic nematicide Furadan® (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) as comparable treatment (Fig 2.5). Three rates of the nematicide were applied (0.5, 1.0 and 2.0 g pot^{-1}).

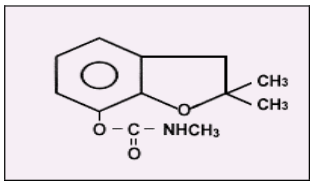


Fig. 2.5: Carbofuran structural formula

2.5 Experimental design

2.5.1 Greenhouse experiment of cyst nematode control strategy - CYNCOS

Enhancing the antiphytopathogenic potential of the soil against cyst nematodes

The investigations were carried out using soil obtained from the field of the Physikalisch-Technische Bundesanstalt (PTB) in Braunschweig, Germany (10° 27′ E, 52° 17′ N). The soil is characterised as strong loamy sand (Sl4) following the German soil classification system (AG Boden, 1994). The soil was mixed with sand (1:1) to be used as experimental soil. The percentages of water content (WC) of the original soil (17 %) and the experimental soil (9 %) were determined according to Schinner et al. (1996) while the maximum water holding capacity (WHC_{max}) of the experimental soil (20 %) was determined according to Stöven (1999). The soil was air-dried and sieved to pass a 2-mm sieve. The chemical characteristics of the soil are summarised in Tab. 2.1.

Tab. 2.1: Chemical characteristics of the experimental soil

Soil parameter	Unit	Soil characteristic				Method
		ES	$ESPM_1$	$ESPM_2$	$ESPM_3$	
PH		5.87	5.85	5.82	5.71	Potentiometric in 0.01 M $CaCl_2$ suspension (Hoffmann, 1991)
C_t	**%**	0.70	1.58	3.27	5.89	Dry combustion; LECO EC-12®, Model 752-100
OSS	**%**	1.21	2.73	5.06	10.15	(Rogasik, 2005)
N_t	**%**	0.070	1.012	0.125	0.177	Kjeldahl (Hoffmann, 1991)
P_{CAL}	**%**	0.0068	0.0076	0.0089	0.0114	Calcium-acetate-lactate (CAL)-extraction method; P was determined by spectrophotometer; K was determined by flame photometry (Schüller, 1969)
K_{CAL}	**%**	0.0067	0.0081	0.0138	0.0248	
Mg	**%**	0.0035	0.0043	0.0074	0.0142	Schachtschabel (1954)

ES = Experimental soil, $ESPM_1$ = Experimental soil mixed with 3 % of commercial potting mixture, $ESPM_2$ = Experimental soil mixed with 12 % of commercial potting mixture. ESPM3 = Experimental soil mixed with 40 % of commercial potting mixture.
OSS = Organic soil substance = C_{org} * 1.724.

The pot experiments were conducted in a greenhouse under controlled conditions. Seedlings of sugar beet plants *c.v.* Tatjana of uniform size were transplanted into 14 cm pots filled with 1.8 kg of experimental soil which was inoculated with 3000 eggs pot^{-1} of the cyst nematode *Heterodera schachtii*. Several treatments were applied to control the population of cyst nematode *H. schachtii* as shown in Tab. 2.2.

Each treatment concentration was carried out with 4 replications, resulting in a total of 20 pots as controls, 24 pots of organic and inorganic fertilisers, 48 pots of fungi and 48 pots of plant extracts which gave a total of 140 pots in the experiment. All pots were arranged in a randomised pattern on a bench in a greenhouse at 20 ± 3 ºC receiving the same horticultural treatment (Fig. 2.6). The applications of treatments were carried out twice, in the beginning of the experiment and one month later except for the treatment of commercial potting mixture which was applied one time in the beginning and the OMF* which applied twice weekly at a rate of 50 ml per pot. Pots were watered daily with deionised water to guaranty an optimal water supply for the growing plants. The experiment lasted 134 day.

* Optimum mineral fertilisation

Tab. 2.2: Experimental design of *Heterodera schachtii* control strategy

	Factor	Abbreviation	Description
Control	Healthy plants	H	Untreated plants (free of plant parasitic nematodes)
	Inoculated plants	I	Untreated plants inoculated with 3000 of cyst nematode *H. Schachtii*
	Carbofuran	CF	Inoculated plants treated by the systemic nematicide Furadan® (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) at three rates (0.5, 1.0 and 2.0 g pot^{-1}) as comparable treatment
Organic and inorganic fertilisers	Commercial potting mixture	PM	Inoculated plants grown in soil mixed with three levels of PM (3, 12 and 40 %).
	Optimum mineral fertilisation	OMF	Inoculated plants treated by the commercial fertiliser Fischer Gartenland® Super Flüssigdünger (N: P_2O_5: K_2O = 6:6:6 + micronutrients B, Cu, Fe, Mn, Mo and Zn). Three rates (50, 100 and 200% of the recommended dose) were applied.
Fungi	*Arthrobotrys oligospora* *Fusarium equiseti* *Trichoderma harzianum* *Verticillium lecanii*	AO FE TH VL	Inoculated plants treated separately by three concentrations (100, 75 and 50%) of each fungal filtrate.
Plant extracts	*Thuja orientalis*	TJOEA TJOH	Inoculated plants treated by three concentrations (1.25, 2.5 and 5%) of *Thuja orientalis* extracts using ethyl acetate (TJOEA) or hexane (TJOH)
	Thevetia neriifolia	TVNEA TVNH	Inoculated plants treated by three concentrations (1.25, 2.5 and 5%) of *Thevetia neriifolia* extracts using ethyl acetate (TVNEA) or hexane (TVNH)

Each treatment concentration was carried out with 4 replications

Fig. 2.6: Greenhouse experiment of the cyst nematode control strategy – CYNCOS

After 3 months the plants were removed from the pots and vegetative measurements as well as soil nematode population and data concerning root embedded stages were determined. Cysts and average number of eggs per cyst were counted. The

rates of nematode reproduction and reductions were calculated. All nematode counts were calculated referring to root and soil fresh weights.

2.5.2 Greenhouse experiment of the citrus nematode control strategy - *CINCOS*

Enhancing the antiphytopathogenic potential of the soil against citrus nematodes

One year old navel orange seedlings of uniform size were planted into 20 cm clay pots filled with 2.5 kg of steam sterilised sandy loam soil and inoculated with 5000 second stage juveniles (J_2) per pot of the citrus nematode *Tylenchulus semipenetrans*. Several treatments have been performed to control the population of *T. semipenetrans* as shown in Tab. 2.3.

Each treatment was replicated four times, resulting in a total of 200 pots. The treatments were arranged in a randomised design on a bench in a greenhouse at 29 ± 5 ºC receiving identical horticulture treatments. The applications of all treatments were carried out twice, in the beginning of the experiment and one month later excepting the treatment of navel orange scions and organic fertilisers, which were applied one time in the beginning. The pots were watered daily to guaranty an optimal water supply for the growing plants. After 4 months, the plants were removed from the pots and data on plant growth response as well as soil nematode population and the root embedded stages were estimated. Egg masses per root and eggs per egg mass were counted. All nematode counts were calculated referring to root and soil fresh weights. Rates of nematode reproduction and reductions were calculated as follows:

$$\% \text{ Nematode reduction} = \frac{\text{Final population of control - Final population of treatment}}{\text{Final population of control}} * 100$$

$$\% \text{ Egg production} = \frac{\text{Eggs per egg mass * No. of egg masses of treatment}}{\text{Eggs per egg mass * No. of egg masses of highest treatment}} * 100$$

Tab. 2.3: Experimental design of *Tylenchulus semipenetrans* control strategy

Factor		Abbreviation	Description
Control	Healthy plants	H	Untreated plants (free of plant parasitic nematodes)
	Inoculated plants	I	Untreated plants inoculated with 3000 J_2 of cyst nematode *T. semipenetrans*
	Carbofuran	CF	Inoculated plants treated by the systemic nematicide Furadan® (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate) at three rates (0.5, 1.0 and 2.0 g pot^{-1}) as comparable treatment
Organic and inorganic fertilisers	Pigeon excrements	PE	Inoculated plants grown in soil mixed with three levels of PE (25, 50 and 100 g pot^{-1})
	Crushed garlic	CRG	Inoculated plants treated by three rates (25, 50 and 100 g pot^{-1}) of SMG
	Ammonium nitrate NH_4NO_3 (33.5% N)	AN	Inoculated plants treated by three rates of AN (5, 10 and 20 g pot^{-1}
Organic and amino acids	Ascorbic acid Salicylic acid	ASC SAL	Inoculated plants treated separately using three concentrations (1, 2 and 4 g L^{-1}) of each organic acid
	Amino acids	AA	Inoculated plants treated by three concentrations of AA (0.5, 1.0 and 2.0 ml L^{-1})
Microbial agents	*Dactylella brochopaga* *Nematoctonus concurrence*	DB NC	Inoculated plants treated separately using three concentrations (100, 75 and 50%) of each fungal filtrate
	Bacillus thuringiensis	BT	Inoculated plants treated by three rates of BT (1, 2 and 4 g pot^{-1})
Resistant rootstocks	*Poncirus trifoliata*	NOPT	Navel orange scion on the resistant rootstock *P. trifoliata* inoculated with citrus nematode
	Citrus volkameriana	NOCV	Navel orange scion on the resistant rootstock *C. volkameriana* inoculated with citrus nematode
Plant extracts	*Thuja orientalis*	TJOEA TJOH	Inoculated plants treated by four concentrations (1.25, 2.5, 5 and 10 %) of *Thuja orientalis* extracts using ethyl acetate (TJOEA) or hexane (TJOH)
	Thevetia neriifolia	TVNEA TVNH	Inoculated plants treated by four concentrations (1.25, 2.5, 5 and 10 %) of *Thevetia neriifolia* extracts using ethyl acetate (TVNEA) or hexane (TVNH)

Each treatment concentration was carried out with 4 replications

2.5.3 Greenhouse experiment of citrus rootstocks susceptibility to the infection with citrus nematodes - ROSSUS

Application of citrus rootstocks as citrus nematode control strategy

One year old cuttings of 6 imported citrus rootstocks of uniform size were obtained from the Department of Horticulture, Faculty of Agriculture at Moshtohor, Zagazig

University, Egypt. These rootstocks were `Cleopatra' mandarin (*Citrus reticulata* Blanco), `Rangpur' lime (*C. limon* Osbeck), sour orange (*C. aurantium* L), rough lemon (*C. jambhiri* Hush), Japanese Bitter Orange (*Poncirus trifoliata* (L.) Raf.) and `Volkamer' lemon (*C. volkameriana* Tanaka).

The rootstock cuttings were cultivated in 20 cm pots filled with steam sterilised sand: loam soil (1:1). The pots were divided in two groups: in the first, each pot was inoculated with 5000 infective stages per pot of citrus nematode *T. semipenetrans*; in the second, each rootstock was planted in soil free of nematodes as a control. Each trial was carried out with 4 replications, which sums up to a total of 72 pots. The pots were arranged in a randomised design on a bench in a greenhouse at 30 ± 4 ºC receiving the same treatments. The inoculum was introduced two months after the cuttings were established and the roots were flushed by pouring the nematode water suspension into 4 holes around the root system. The pots were watered daily to ensure an optimal water supply for the growing plants. Seedlings were removed 6 months after inoculation. Data of vegetative measurements were estimated. Soil and roots of each rootstock were examined, including the count of 2nd stage juveniles in soil, egg masses per root, eggs per egg mass, and immature and mature females per root. The rate of nematode build-up (Pf Pi^{-1}), percentage of egg production and eggs per g root were calculated. All nematode counts were calculated referring to root and soil fresh weights. The rate of penetration and potential reproductive index (PRI) were calculated according to the following formulas:

$$\text{Rate of nematode penetration} = \frac{\text{Total counts of nematodes in roots}}{\text{Initial nematode inoculation}} * 100$$

$$\text{Population reproduction index} = \frac{\text{Nematode final population of rootstock}}{\text{Nematode final population of highest rootstock}} * 100$$

2.5.4 Field experiment with citrus trees – FECIT

Timing of application

Population dynamics of the citrus nematodes *Tylenchulus semipenetrans* were studied from June 2002 to June 2004, monitoring the seasonal cycles of high populations to determine the most suitable application time of the nematode control strategies. Ten citrus trees at the experimental orchards of the Faculty of Agriculture (Moshtohor),

Zagazig University, Egypt were chosen to determine the population peaks of citrus nematodes. At monthly intervals, soil samples at 20 and 50 cm depth were collected from each tree after removing the superficial layer (5 cm) of soil.

The samples were transferred to the laboratory in labelled polyethylene bags and processed soon after collection. The numbers of free second stage juveniles were estimated. The curve of seasonal fluctuation was drawn to show the population peaks.

2.6 Statistical analysis

The data obtained from all designed experiments were statistically analysed according to the SPSS software package version 12 (SPSS, 2003). The differences between means were tested using Tukey's multiple tests at the 5 % significance level.

3 Results

The cyst nematode *Heterodera schachtii* and the citrus nematode *Tylenchulus semipenetrans* were used as indicators of soil quality and health.

In all presented data, the nematicide Carbofuran was used as comparable treatment. Because of the differences in the concentrations and compositions between treatments, each treatment was applied in three doses (low, medium and high). These levels were previously described in details at pages 14 and 16 in the chapter of Materials and Methods.

3.1 Enhancing the antiphytopathogenic potential of soil against the sugar beet cyst nematode *Heterodera schachtii*

3.1.1 Organic and inorganic fertilisers

The potential of commercial potting mixture (70 % water content) as an organic amendment, OMF* as an inorganic fertiliser and the effect of the nematicide Carbofuran on *H. schachtii* reproductivity and sugar beet growth response were studied and evaluated.

The illustrated data in Tab. 3.1 reveal that all treatments at all levels significantly suppressed the target nematode criteria in both soil and root compared to the control. Differences in nematode suppression were noticeable among treatments and/or doses. For instance, all applied levels resulted in high reductions of all nematode criteria and exhibited acute decreases in eggs per cyst, especially at the rate of 40 % with 209 eggs $cyst^{-1}$, followed by the liquid fertiliser OMF at the recommended dose (100 %) with 212 eggs $cyst^{-1}$. Significant reductions in the numbers of cysts, population of 2^{nd} stage juveniles (J_2), immature stages and mature females were observed among all treatments even at their lowest rates.

The addition of commercial potting mixture 40 % (439 g pot^{-1}) recorded the highest rates of nematode suppression even when compared to the nematicide at its highest concentration. Compared to the control, the populations of 2^{nd} stage juveniles, immature stages and mature females were reduced to 552, 52 and 1 individuals, respectively.

The OMF was highly successful in reducing nematode counts in both soil and roots especially when used at the recommended concentration. The populations of the 2^{nd} stage juveniles were reduced to 631 J_2 100 g^{-1} soil. Furthermore, the immature stages and mature females were reduced to 58 and 2 individuals g^{-1} root, respectively.

* Optimum mineral fertilisation

Tab. 3.1: Reproductivity of *Heterodera schachtii* infecting sugar beet seedlings as influenced by addition of organic and inorganic fertilisers

Treatment	Dose	Soil (100 cm^3)		Roots (1 g)	
		Eggs $cyst^{-1}$	J_2	Imm. stages	Mature Females
Commercial potting mixture (%)	3	272^{ab}	758^{b}	71^{b}	2^{b}
	12	260^{ab}	587^{b}	55^{b}	2^{b}
	40	209^{b}	552^{b}	52^{b}	1^{b}
OMF (%)	50	279^{ab}	758^{b}	68^{b}	3^{b}
	100	212^{b}	631^{b}	58^{b}	2^{b}
	200	228^{b}	724^{b}	63^{b}	2^{b}
Carbofuran (g pot^{-1})	0.5	289^{ab}	684^{b}	64^{b}	2^{b}
	1.0	229^{b}	655^{b}	53^{b}	1^{b}
	2.0	224^{b}	564^{b}	51^{b}	0^{b}
Control		350^{a}	1723^{a}	166^{a}	6^{a}

J_2 = Second stage juvenile, Imm. stages = Immature stages
Control = Plants inoculated with nematodes without any treatment.
Commercial potting mixture was mixed with soil in three levels 3 % (55 g pot^{-1}), 12 % (199 g pot^{-1}), and 40 % (439 g pot^{-1}). Fertiliser was applied as 50, 100 and 200 % of the recommended dose.
Mean values followed by the same letters in column are not significantly different by Tukey's test at 0.05 levels.

The count of cysts is the most important factor that is a reliable indicator of the nematode population. The relationship between the respective treatments and number of cysts was drawn in Fig. 3.1. It was observed that all treatments achieved high reductions in the number of cysts. Commercial potting mixture at a rate of 40 % recorded the highest effectiveness in reducing the number of cysts with 18 cysts followed by the rate of 12 % with 23 cysts. OMF* at the recommended dose (100 %) followed the commercial potting mixture and resulted in 24 cysts. Furthermore, both commercial potting mixture doses and the recommended dose of OMF exceeded the nematicide Carbofuran even at its highest doses in their cyst reduction. There was a clear correlation between the concentration of the treatments and cyst reduction. For instance, the effectiveness of commercial potting mixture in reducing the number of cysts increased with increasing concentration, while in case of OMF the highest effectiveness was achieved at the recommended dose (100 %). Its ability decreased at higher or lower doses.

* Optimum mineral fertilisation

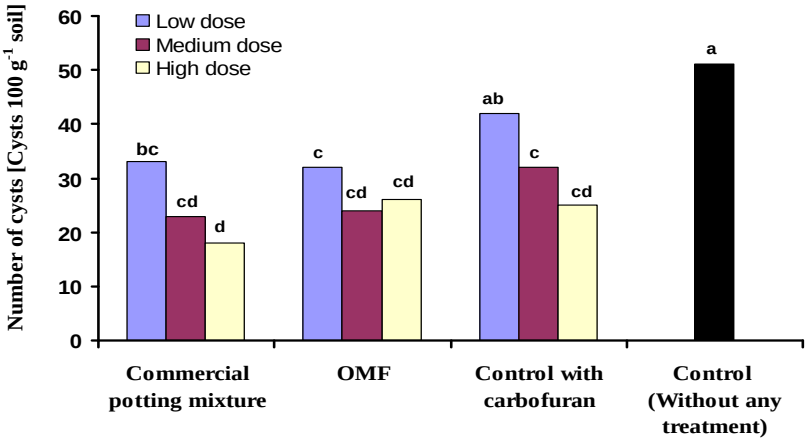


Fig. 3.1: Number of cysts of *Heterodera schachtii* infecting sugar beet seedlings as influenced by organic and inorganic fertilisers

Concerning the percentage of nematode reduction (Fig. 3.2) the commercial potting mixture achieved the highest nematode reduction percentage (67.9) at an application rate of 40 %, while the highest concentration of the nematicide Carbofuran achieved 66.8 %. OMF* at the recommended dose was most effective in nematode reduction with 63.1 %.

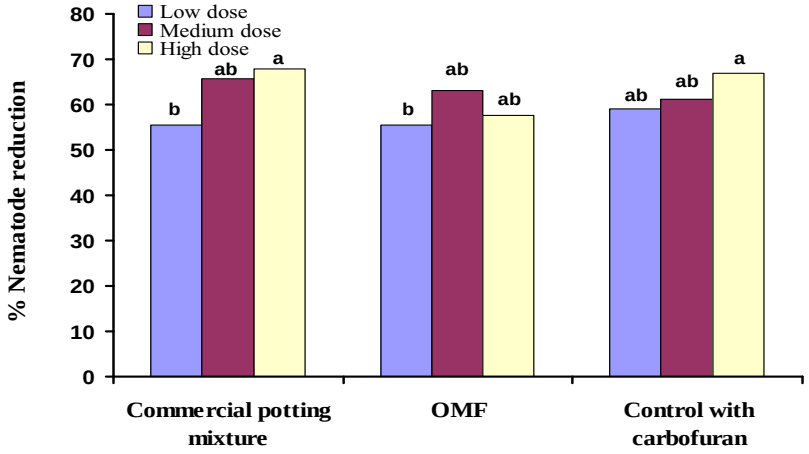


Fig. 3.2: Population reductions of *Heterodera schachtii* as influenced by organic and inorganic fertilisers

The nematode build-up (Pf Pi^{-1}) was calculated and is illustrated in Fig. 3.3. It was observed that the respective treatments showed variation in nematode build-up. It ranged between 4.7 fold with commercial potting mixture 3 % and OMF 50 % to 3.4 fold with

* Optimum mineral fertilisation

commercial potting mixture 40 %. All treatments and doses achieved a significant reduction in nematode build-up when compared to the control (10.6 fold).

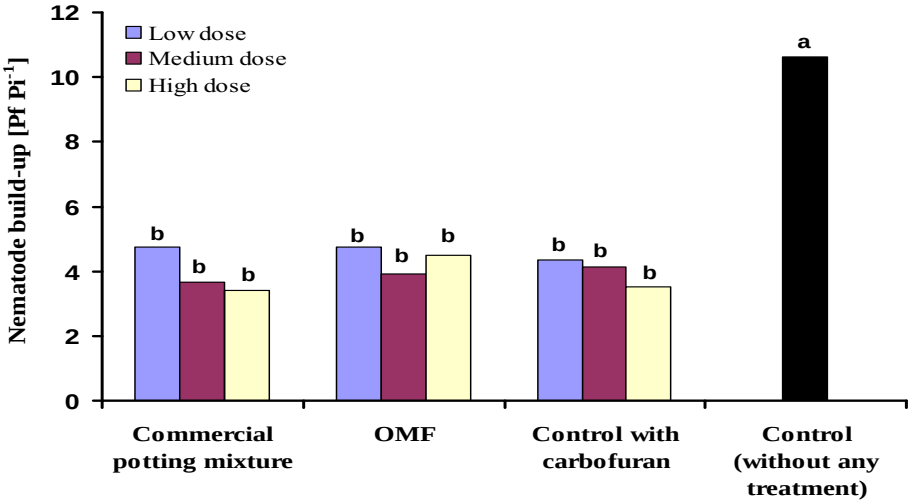


Fig. 3.3: Population build-up of *Heterodera schachtii* as influenced by organic and inorganic fertilisers

Data of sugar beet seedlings growth response are listed in Tab. 3.2. The data indicated to positive relations between all fertiliser treatments and plant growth parameters. It was observed that most of the treatment doses recorded increases in plant growth criteria even when compared to the healthy plants.

According to the shoot parameters, all levels of fertilisers were associated with increases in plant height as well as fresh and dry weight. Significant increases were observed especially at the high application levels. The maximum shoot height (30.5 cm) as compared to the control (11.9 cm) was achieved when using commercial potting mixture at a rate of 40 %. At the same dosage, the fresh and dry weights were increased more than 5 times. Furthermore, commercial potting mixture at 40 and 12 % exceeded the healthy plants in all shoot measurements. OMF* at the recommended concentration also achieved significant increases in shoot growth at all applied concentrations compared to the control with 20.9 cm in plant height, and 25.1 and 4.1 g in fresh and dry weight, respectively.

In the case of root growth parameters, no significant effects were observed in root length. Clear proportional effects were observed between materials/doses and root length except for the treatment of commercial potting mixture. For instance, OMF showed a positive response, which increased steadily by increasing the dosage. The highest increase as compared to the control was recorded at a concentration of 200 %, resulting in a length

* Optimum mineral fertilisation

of 62 cm. On the other hand, Carbofuran achieved increases in root length at all doses, however, these increases declined at higher dosages. The highest root length was recorded using Carbofuran at a rate of 0.5 g pot^{-1}, resulting in a length of 63.3 cm. According to the fresh and dry weight, significant effects were noticeable at all applied rates of the fertilisers compared to the control. Commercial potting mixture at a level of 40 % recorded increases of more than 11 times the root fresh weight and more than nine times the root dry weight, compared to the control. Furthermore, at the same level, high increases in fresh and dry weights were achieved to more than double when compared to the non-inoculated plants (healthy plants). OMF* achieved the highest increases at a concentration of 100 % resulting in 29.9 and 6.7 g in fresh and dry weight, respectively. No significant differences were noticed in root fresh and dry weights when using Carbofuran at all doses.

Tab. 3.2: Growth response of sugar beet seedlings infected with *Heterodera schachtii* as influenced by the addition of organic fertilisers

Treat.		Shoot			Root		
		Height (cm)	Fresh wt. (g)	Dry wt. (g)	Length (cm)	Fresh wt. (g)	Dry wt. (g)
Commercial potting mixture (%)	3	19.1^{cd}	11.8^{c}	2.0^{de}	42.5^{a}	19.2^{cd}	3.9^{cd}
	12	24.8^{b}	22.8^{b}	3.9^{bc}	46.6^{a}	40.1^{b}	8.7^{b}
	40	30.5^{a}	44.5^{a}	7.3^{a}	48.8^{a}	67.6^{a}	14.9^{a}
OMF (%)	50	20.6^{bc}	21.7^{b}	3.5^{bc}	51.8^{a}	25.2^{c}	6.3^{bc}
	100	20.9^{bc}	25.1^{b}	4.1^{b}	57.8^{a}	29.9^{bc}	6.7^{bc}
	200	22^{bc}	24.6^{b}	3.9^{bc}	62^{a}	26.7^{bc}	6.1^{bc}
Carbofuran (g pot^{-1})	0.5	9.4^{f}	4.6^{c}	0.8^{f}	63.3^{a}	5.5^{d}	1.3^{d}
	1.0	12.9^{ef}	7.7^{c}	1.3^{ef}	56^{a}	7^{d}	1.6^{d}
	2.0	15.5^{de}	9.1^{c}	1.5^{ef}	52.5^{a}	6.9^{d}	1.5^{d}
Healthy plants		23.9^{b}	20.8^{b}	2.8^{cd}	36.8^{a}	26.9^{bc}	7.4^{bc}
Control		11.9^{ef}	6.5^{c}	1.1^{ef}	49^{a}	5.4^{d}	1.4^{d}

Treat. = Treatment, wt = weight, Commercial potting mixture was mixed with soil in three levels 3 % (55 g pot^{-1}), 12 % (199 g pot^{-1}), and 40 % (439 g pot^{-1}). Fertiliser was used as 50, 100 and 200 % of the recommended dose. Control = Plants inoculated with nematodes without any treatment, Healthy plants= Plants free of nematodes and any treatment.
Mean values followed by the same letters in column are not significantly different by Tukey's test at 0.05 levels.

* Optimum mineral fertilisation

3.1.2 Microbial agents

Filtrates of *Arthrobotrys oligospora*, *Fusarium equiseti, Trichoderma harzianum* and *Verticillium lecanii* treated as soil drench were applied in three different levels to test their effectiveness against the target nematode pest, *H. schachtii* infecting sugar beet seedlings as well as their effect on plant growth response under greenhouse conditions.

Data in Tab. 3.3 indicate that all fungi filtrates applied impaired nematode reproductivity. All the treatments significantly reduced the numbers of mature, immature stages, J_2 in soil and consequently the nematode build-up comparing to the control. Furthermore, a stark reduction in the counts of cysts and eggs per cyst was noticed. Differences in nematode suppression were noticeable among treatments and/or doses. The inhibition of nematode counts and fecundity was increased steadily by increasing the filtrate concentration. The highest reduction in nematode parameters was achieved when fungi filtrates were applied at their highest dose without dilutions (100 %). Furthermore, the undiluted filtrates exceeded the effect of nematicide Carbofuran even at its highest dose.

The antagonistic fungus *F. equiseti* at its maximum dose achieved the strongest effect on *H. schachtii* population and fecundity with 280 eggs $cyst^{-1}$, 573 J_2 100 g^{-1} soil, 53 immature stages and 1 mature female g^{-1} root. Inimical effects also were achieved by fungi filtrates of *V. lecanii* and *A. oligospora* followed by *T. harzianum.*

Tab. 3.3: Reproductivity of *Heterodera schachtii* infecting sugar beet seedlings as influenced by addition of microbial agents

Treatment	Dose (%)	Soil (100 cm^3)		Roots (1 g)	
		Eggs $cyst^{-1}$	J_2	Imm. stages	Mature Females
Arthrobotrys oligospora	**50**	286^{ab}	766^{b}	74^{b}	2^{bcde}
	75	304^{ab}	708^{b}	61^{b}	2^{cde}
	100	275^{ab}	675^{b}	55^{b}	1^{cde}
Fusarium equiseti	**50**	294^{ab}	730^{b}	67^{b}	2^{bcde}
	75	298^{ab}	725^{b}	58^{b}	2^{cde}
	100	280^{ab}	573^{b}	53^{b}	1^{cde}
Trichoderma harzianum	**50**	302^{ab}	996^{b}	102^{b}	4^{ab}
	75	275^{ab}	771^{b}	82^{b}	3^{bcd}
	100	233^{b}	693^{b}	70^{b}	2^{bcde}
Verticillium lecanii	**50**	273^{ab}	830^{b}	83^{b}	4^{bc}
	75	240^{ab}	758^{b}	69^{b}	2^{bcde}
	100	207^{b}	670^{b}	62^{b}	2^{cde}
Carbofuran (g pot^{-1})	**0.5**	289^{ab}	684^{b}	64^{b}	2^{bcde}
	1.0	229^{b}	655^{b}	53^{b}	1^{de}
	2.0	224^{b}	564^{b}	51^{b}	0^{e}
Control		350^{a}	1723^{a}	166^{a}	6^{a}

J_2 = Second stage juvenile, Imm. stages = Immature stages, Control = Plants inoculated with nematodes without any treatment. Each fungus was applied as 50, 75 and 100 % of filtrate concentration.
Mean values followed by the same letters in column are not significantly different by Tukey's test at 0.05 levels.

The number of cysts in soil (Fig. 3.4) was decreased strongly compared to the control or Carbofuran at its highest concentration, due to the nematicidal effect of the concerned filtrates especially when used at their highest rates. For instance, filtrates of *F. equiseti* recorded the maximum effect in reducing numbers of cysts with 18 cysts 100 g^{-1} soil followed by *V. lecanii* and *A. oligospora* with 20 cysts 100 g^{-1} soil and finally *T. harzianum* with 21 cysts 100 g^{-1} soil.

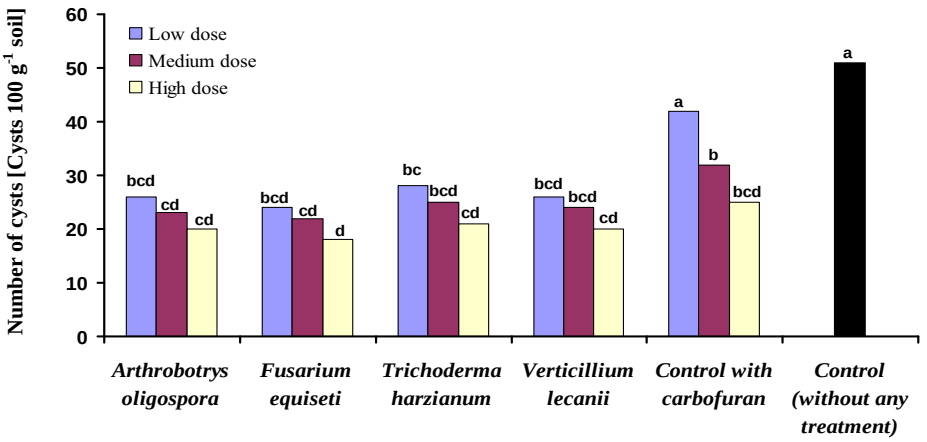


Fig. 3.4: Number of cysts of *Heterodera schachtii* infecting sugar beet seedlings as influenced by fungi filtrates

High percentages were achieved in nematode reduction as illustrated in Fig. 3.5. The highest reduction, 67 % was achieved when using the nematicide Carbofuran at its highest dose (2.0 g pot^{-1}). Resembling result was recorded using filtrates of *F. equiseti* at the maximum dose with 67 %. Filtrates of *V. lecanii* and *A. oligospora* followed by *T. harzianum* recorded reductions in nematode populations with 61.1, 60.8 and 59.8 %, respectively, at their highest doses.

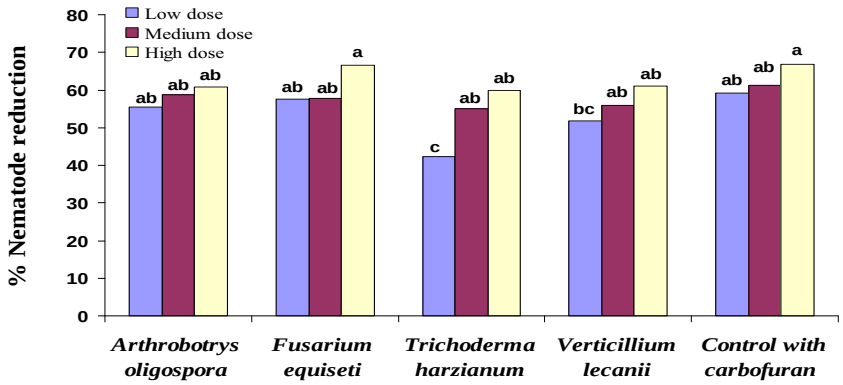


Fig. 3.5: Population reductions of *Heterodera schachtii* as influenced by fungi filtrates

The rates of nematodes build-up (Fig. 3.6) were significantly decreased with treatment by fungi filtrates at all doses compared to the control. The highest reduction in nematode build-up was achieved using the highest filtrate doses. The lowest build-up of *H. schachtii* was achieved by the filtrates of *F. equiseti* and the nematicide Carbofuran at their maximal doses, resulting in 3.5 fold followed by *V. lecanii*, *A. oligospora* and finally *T. harzianum*. No significant differences were noticed between the treatments.

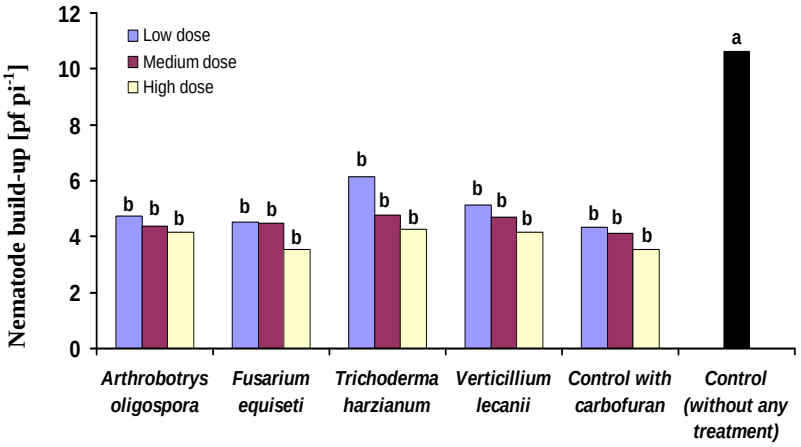


Fig. 3.6: Population build-up of *Heterodera schachtii* as influenced by fungi filtrates

Data of sugar beet seedlings growth response as influenced by fungi filtrates are listed in Tab. 3.4. The obtained data displayed positive relations between all the treatments and plant growth parameters. It was noticed that most treatment doses recorded increases in plant growth criteria.

According to the shoot parameters, increases in the plant height as well as the fresh and dry weights were observed especially at higher doses. The maximum increases compared to the control were achieved by using fungi filtrates at their maximum rates. According to plant height, the filtrate of *A. oligospora* at the highest dose achieved significant increase compared to the control with 21.5 cm, followed by *F. equiseti*, *V. lecanii*, *T. harzianum* and finally Carbofuran at their highest doses with 19.1, 18.6, 17.5 and 15.5 cm, respectively. Furthermore, at the highest doses of fungi filtrates, fresh and dry weights were increased compared to the control resulting in 13 and 2.1 g, respectively, for *F. equiseti*. Fresh and dry weights were 12.9 and 2.3 g, respectively for *T. harzianum*, followed by *A. oligospora*, *V. lecanii* and finally Carbofuran.

On the basis of the root growth parameters, no clear proportional effects were observed between materials/doses and the root length except for Carbofuran, which showed increases in root length with decreasing dosage. Its highest value compared to the control was recorded at a dose of 0.5 g pot^{-1}, with a length of 63.3 cm. However, clear proportional effects were observed among materials/doses and fresh and dry weight; all doses yielded increases in mass with a positive correlation between parameters. The highest values when compared to the control were recorded at the highest doses of filtrates. For instance, *A. oligospora* and *F. equiseti* at their highest dose recorded fresh weights of

more than twice those of control, with 16.6 and 17.5 g, respectively. At the same dose they recorded 4 and 3.6 g in dry weight, respectively, followed by *V. lecanii*, *T. harzianum* and finally Carbofuran.

Tab. 3.4: Growth response of sugar beet seedlings infected with *Heterodera schachtii* as influenced by the addition of microbial agents

Treat.	Dose (%)	Shoot			Root		
		Height (cm)	Fresh wt. (g)	Dry wt. (g)	Length (cm)	Fresh wt. (g)	Dry wt. (g)
Arthrobotrys oligospora	**50**	12.9^{efg}	6^{d}	1.1^{e}	45.3^{a}	7.1^{def}	1.6^{d}
	75	15.9^{cde}	8.7^{bcd}	1.3^{cde}	59.5^{a}	9.5^{cdef}	2.2^{bcd}
	100	21.5^{ab}	12.6^{b}	2.1^{abc}	53.3^{a}	16.6^{bc}	4^{b}
Fusarium equiseti	**50**	12.3^{efg}	6.2^{d}	1.1^{e}	58.5^{a}	6.5^{def}	1.5^{d}
	75	14^{def}	8.5^{bcd}	1.3^{de}	43.4^{a}	10.6^{bcdef}	2.4^{bcd}
	100	19.1^{bc}	13^{b}	2.1^{abc}	43.1^{a}	17.5^{b}	3.6^{bc}
Trichoderma harzianum	**50**	11.9^{efg}	6.8^{cd}	1.2^{de}	47^{a}	8.4^{def}	2^{cd}
	75	14^{def}	8.3^{bcd}	1.5^{cde}	45.8^{a}	7.3^{def}	1.7^{d}
	100	17.5^{bcd}	12.9^{b}	2.3^{ab}	52^{a}	13.4^{bcde}	2.8^{bcd}
Verticillium lecanii	**50**	11.4^{fg}	6.5^{cd}	1.1^{e}	39.5^{a}	6.1^{def}	1.4^{d}
	75	13.1^{efg}	8.3^{bcd}	1.4^{cde}	48.8^{a}	8.6^{def}	1.9^{cd}
	100	18.6^{bc}	11.9^{bc}	2^{bcd}	37.3^{a}	13.6^{bcd}	3^{bcd}
Carbofuran (g pot^{-1})	**0.5**	9.4^{g}	4.6^{d}	0.8^{e}	63.3^{a}	5.5^{ef}	1.3^{d}
	1.0	12.9^{efg}	7.7^{bcd}	1.3^{de}	56^{a}	7^{def}	1.6^{d}
	2.0	15.5^{cde}	9.1^{bcd}	1.5^{cde}	52.5^{a}	6.9^{def}	1.5^{d}
Healthy plants		23.9^{a}	20.8^{a}	2.8^{a}	36.8^{a}	26.9^{a}	7.4^{a}
Control		11.9^{efg}	6.5^{d}	1.1^{e}	49^{a}	5.4^{f}	1.4^{d}

Treat. = Treatment, wt. = weight, Control = Plants inoculated with nematodes without any treatment. Healthy plants= Plants free of nematodes and any treatment. Each fungus was applied as 50, 75 and 100 % of filtrate concentration.
Mean values followed by the same letters in column are not significantly different by Tukey's test at 0.05 levels.

3.1.3 Plant extracts

Dilutions of *Thevetia neriifolia* and *Thuja orientalis,* extracted by the solvents ethyl acetate or hexane and treated as soil drench, were used at three different levels to test their effectiveness against the target nematode pest, *Heterodera schachtii,* in sugar beet seedlings under greenhouse conditions.

The effects of the ethyl acetate plant extracts on the cyst nematode population and fecundity are summarised in Tab. 3.5 and shown in Fig. 3.7 - Fig. 3.8 - Fig. 3.9. Generally, the plant extracts using the solvent ethyl acetate clearly impaired nematode population and reproductivity. All the treatments exhibited significant reduction of eggs per cyst, count of J_2, immature stages and mature females compared to the control. Furthermore, differences in nematode suppression were noticed between treatments and/or doses. In both plant extracts, the inhibition of nematode counts and fecundity increased steadily by increasing the extract concentration until reaching their maximum effectiveness at a concentration of 5 %.

Thuja orientalis extracts achieved a higher reduction in all nematode parameters at all concentration levels compared to extracts of *Thevetia neriifolia* except for the count of eggs per cyst. *Thevetia neriifolia* at a dose of 5 % achieved 227 eggs $cyst^{-1}$ and exceeded the effect of *Thuja orientalis* at its highest dose.

A concentration of 5 % was optimal in reducing nematode count and fecundity for both extracts. At this concentration, *Thuja orientalis* yielded the highest reductions in all nematode criteria compared to the control with 706 J_2 100 g^{-1} soil, 71 and 3 individuals g^{-1} root of the immature stages and mature females, respectively. *Thevetia neriifolia* achieved its highest results with 726 J_2 100 g^{-1} soil, 75 and 3 individuals g^{-1} root of the immature stages and mature females, respectively.

No significant differences were observed between plant extracts and Carbofuran in the count of J_2 or number of immature individuals.

Tab. 3.5: Reproductivity of *Heterodera schachtii* infecting sugar beet seedlings as influenced by addition of ethyl acetate botanical extracts

Plant extract	Conc.	Soil (100 cm^3)		Roots (1 g)	
		Eggs $cyst^{-1}$	J_2	Imm. stages	Mature Females
***Thevetia neriifolia* (%)**	**1.25**	281^{ab}	849^{b}	91^{b}	3^{b}
	2.5	254^{ab}	804^{b}	88^{b}	4^{b}
	5	227^{b}	726^{b}	75^{b}	3^{bc}
***Thuja orientalis* (%)**	**1.25**	280^{ab}	800^{b}	83^{b}	3^{bc}
	2.5	269^{ab}	753^{b}	76^{b}	3^{b}
	5	230^{ab}	706^{b}	71^{b}	3^{bc}
Carbofuran (g pot^{-1})	**0.5**	289^{ab}	684^{b}	64^{b}	2^{bcd}
	1	229^{b}	655^{b}	53^{b}	1^{cd}
	2	224^{b}	564^{b}	51^{b}	0^{d}
Control		350^{a}	1723^{a}	166^{a}	6^{a}

J_2 = Second stage juvenile, Imm. stages = Immature stages, Control = Plants inoculated with nematodes without any treatment.
The plant extracts were added to soil as three dilutions (1.25, 2.5, and 5%) of crude extract.
Mean values followed by the same letters in column are not significantly different by Tukey's test at 0.05 levels.

The number of cysts in soil as shown in Fig. 3.7 decreased strongly when compared to the control due to the nematicidal effect of the concerned plant extracts especially when used at their highest concentrations. For instance, extract of *Thuja orientalis* and *Thevetia neriifolia* were most effective at reducing number of cysts, to 28 and 29 cysts 100 g^{-1} soil, when applied at the highest concentration.

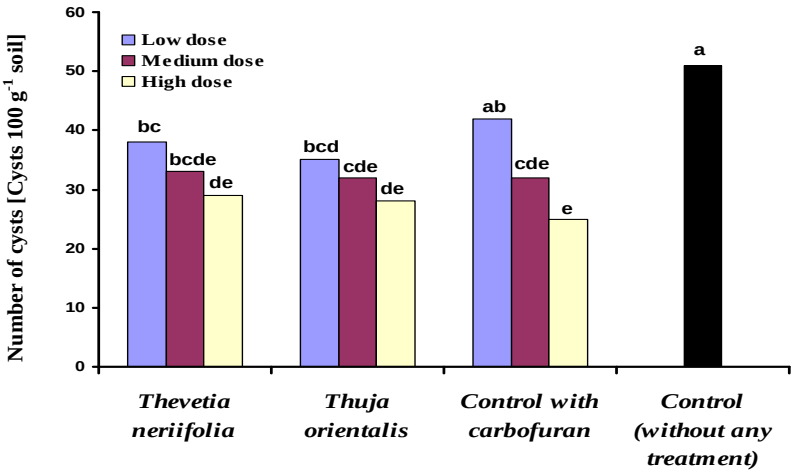


Fig. 3.7: Number of cysts of *Heterodera schachtii* infecting sugar beet seedlings as influenced by ethyl acetate extracts of plants

High percentages were observed in nematode reduction as illustrated in Fig. 3.8. The highest percentage (66.8) was achieved using the nematicide Carbofuran at its highest

dose (2.0 g pot^{-1}). Extracts of *Thuja orientalis* and *Thevetia neriifolia* at their highest doses achieved nematode reduction with 58.6 and 57.5 %, respectively.

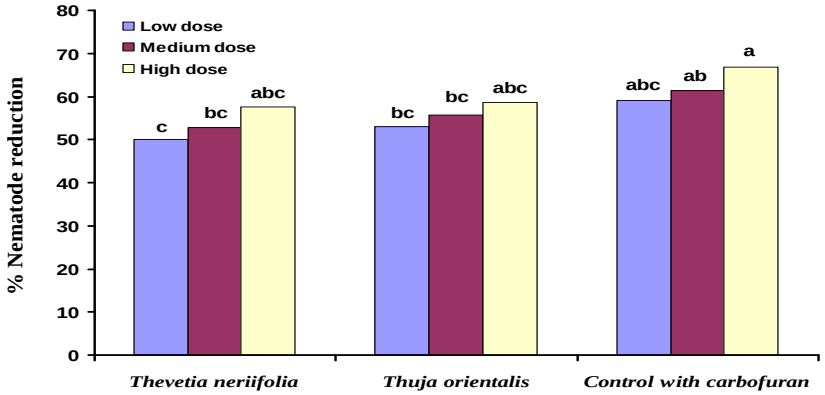


Fig. 3.8: Population reductions of *Heterodera schachtii* as influenced by ethyl acetate extracts of plants

Great reduction in nematode build-up (Fig. 3.9) was observed after treatment with plant extracts at all doses. The highest reduction in nematode build-up was achieved when using the highest treatment doses. The minimum build-up values of *H. schachtii* were achieved by the nematicide Carbofuran even at its lowest doses, followed by extracts of *Thuja orientalis* and *Thevetia neriifolia* at their highest concentrations with 4.40 and 4.53 fold, respectively. No significant differences were detected among treatments.

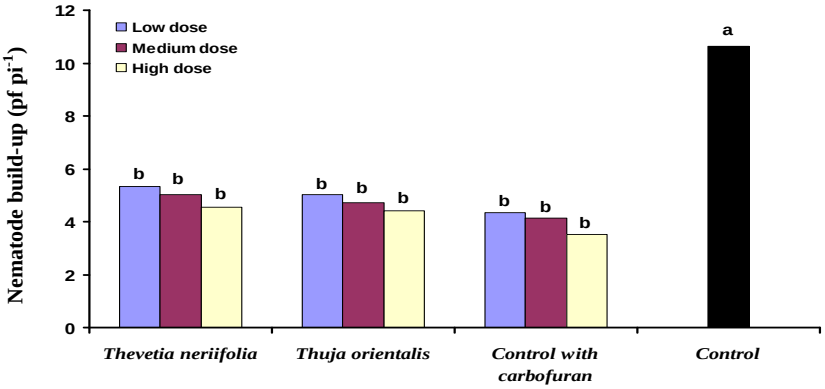


Fig. 3.9: Population build-up of *Heterodera schachtii* as influenced by ethyl acetate extracts of plants

Data of sugar beet seedlings growth response as influenced by ethyl acetate extracts of plants are shown in Tab. 3.6. The data indicated positive relationship between the treatments and plant growth parameters. It was observed that most treatment doses recorded clear increases in plant growth criteria.

In the case of the shoot growth parameters, increases in plant height as well as fresh and dry weight were observed primarily at high doses. The maximum increments as compared to the control were achieved when using plant extracts at their highest rates. According to the plant height, the treatment achieving the greatest increase as compared to the control was Carbofuran at its highest concentration with 15.5 cm, followed by *Thuja orientalis* and finally *Thevetia neriifolia* at their highest doses with 14.9 and 13.8 cm, respectively. Furthermore, at the highest doses of plant extracts, fresh and dry weights of the plants were also increased compared to the control. For instance, extracts of *Thevetia neriifolia* yielded 11.4 and 2 g, respectively followed by *Thuja orientalis* with 10.6 and 1.8 g, respectively while the comparable nematicide Carbofuran achieved lower fresh and dry weights even at its highest dose with 9.1 and 1.5 g, respectively.

According to the root growth parameters, proportional effects were observed between materials/doses and the root length except for the application of *Thevetia neriifolia* extracts. Carbofuran and extracts of *Thuja orientalis* showed an increase in root length with decreasing dosage. The greatest root length increases as compared to the control were achieved at the lowest doses, yielding 43 and 44.3 cm, respectively. On the other hand, increases in root fresh and dry weight at all concentrations of plant extracts were observed. Data yielded positive correlation between fresh and dry weight and plant extract concentration. The highest increases compared to the control were achieved at the highest concentration of each plant extract. For instance, *Thevetia neriifolia* at a concentration of 5 % yielded 13.6 and 3.4 g in fresh and dry weights, respectively, while extracts of *Thuja orientalis* at the same concentration yielded 13.8 and 3.2 g, respectively, followed by Carbofuran at 1.0 g pot^{-1} with 7 and 1.6 g, respectively.

Tab. 3.6: Growth response of sugar beet seedlings infected with *Heterodera schachtii* as influenced by the addition of ethyl acetate extracts of plants

Plant extr.	Conc.	Shoot			Root		
		Height (cm)	Fresh wt. (g)	Dry wt. (g)	Length (cm)	Fresh wt. (g)	Dry wt. (g)
Thevetia neriifolia (%)	**1.25**	10.1^{e}	6.7^{de}	1.2^{cde}	54^{a}	7.1^{c}	1.8^{cd}
	2.5	12.3^{bcde}	6.8^{de}	1.2^{cde}	55.5^{a}	7.1^{c}	1.7^{d}
	5	13.8^{bcd}	11.4^{b}	2^{b}	43^{a}	13.6^{b}	3.4^{b}
Thuja orientalis (%)	**1.25**	10.9^{de}	6.1^{de}	1^{de}	56^{a}	7^{c}	1.6^{d}
	2.5	11^{de}	6.9^{de}	1.2^{cde}	54^{a}	8.2^{c}	1.9^{cd}
	5	14.9^{bc}	10.6^{bc}	1.8^{bc}	44.3^{a}	13.8^{b}	3.2^{bc}
Carbofuran (g pot^{-1})	**0.5**	9.4^{e}	4.6^{e}	0.8^{e}	63.3^{a}	5.5^{c}	1.3^{d}
	1	12.9^{bcde}	7.7^{cde}	1.3^{cde}	56^{a}	7^{c}	1.6^{d}
	2	15.5^{b}	9.1^{bcd}	1.5^{bcd}	52.5^{a}	6.9^{c}	1.5^{d}
Healthy plants		23.9^{a}	20.8^{a}	2.8^{a}	36.8^{a}	26.9^{a}	7.4^{a}
Control		11.9^{cde}	6.5^{de}	1.1^{de}	49^{a}	5.4^{c}	1.4^{d}

Plant extr. = Plant extract, Conc. = Concentration, wt = weight, Control = Plants inoculated with nematodes without any treatment, Healthy plants= Plants free of nematodes and any treatment.
The plant extracts were added to soil as three dilutions (1.25, 2.5, and 5%) of crude extract.
Mean values followed by the same letters in column are not significantly different by Tukey's test at 0.05 levels.

The effects of the ethyl acetate plant extracts on the cyst nematode population and fecundity are summarised in Tab. 3.7 and Fig. 3.10 - Fig. 3.11 - Fig. 3.12. The data in Tab. 3.7 revealed that the dilutions of *Thevetia neriifolia* and *Thuja orientalis* extracted by the solvent hexane and treated as soil drench impaired the nematode counts and reproductivity. All treatments showed significant reductions of the eggs per cyst, numbers of J_2, count of immature stages and mature females. Furthermore, differences in nematode suppression were noticeable between treatments and/or doses. In both plant extracts the nematode count and fecundity decreased steadily by increasing the extract concentration until reaching the maximal effectiveness in nematode reduction at a concentration of 5 %. *Thuja orientalis* achieved greater reductions in nematode parameters at all concentration levels compared to *Thevetia neriifolia.*

A concentration of 5 % was the optimal concentration in reducing nematode count and fecundity for both extracts. At this concentration, *Thuja orientalis* yielded a high

reduction of all nematode criteria with 231, 735, 77 and 3 eggs cyst^{-1}, J_2, immature stages and mature females, respectively. *Thevetia neriifolia* recorded its maximum rates with 240, 764, 81 and 3 eggs cyst^{-1}, J_2, immature stages and mature females, respectively.

No significant differences were observed between the concerned plant extracts and Carbofuran in the number of second stage juveniles (J_2).

Tab. 3.7: Reproductivity of *Heterodera schachtii* infecting sugar beet seedlings as influenced by addition of hexane botanical extracts

Plant extract	Conc.	Soil (100 cm^3)		Roots (1 g)	
		Eggs cyst^{-1}	J_2	Imm. stages	Mature Females
***Thevetia neriifolia* (%)**	**1.25**	314^{ab}	970^{b}	120^{ab}	4^{ab}
	2.5	272^{ab}	827^{b}	110^{abc}	4^{bc}
	5	240^{b}	764^{b}	81^{bc}	3^{bcd}
***Thuja orientalis* (%)**	**1.25**	277^{ab}	859^{b}	105^{abc}	3^{bc}
	2.5	245^{b}	789^{b}	97^{bc}	3^{bc}
	5	231^{b}	735^{b}	77^{bc}	3^{bcd}
Carbofuran (g pot^{-1})	**0.5**	289^{ab}	684^{b}	64^{bc}	2^{cde}
	1	229^{b}	655^{b}	53^{c}	1de
	2	224^{b}	564^{b}	51^{c}	0^{e}
Control		350^{a}	1723^{a}	166^{a}	6^{a}

J_2 = Second stage juvenile, Imm. stages = Immature stages, Control = Plants inoculated with nematodes without any treatment.
The plant extracts were added to soil as three dilutions (1.25, 2.5, and 5%) of crude extract.
Mean values followed by the same letters in column are not significantly different by Tukey's test at 0.05 levels.

The number of cysts in the soil (Fig. 3.10) was decreased strongly when compared to the control due to the nematicidal effect of the concerned plant extracts, primarily when used at highest concentrations. For instance, herbal extracts of *Thuja orientalis* and *Thevetia neriifolia* at highest concentration yielded maximal effects in reducing the number of cysts with 29 and 32 cysts 100 g^{-1} soil, respectively.

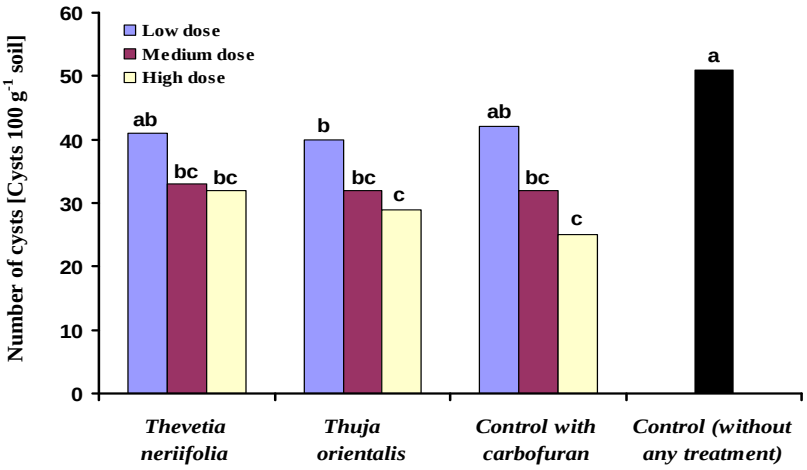


Fig. 3.10: Number of cysts of *Heterodera schachtii* infecting sugar beet seedlings as influenced by hexane extracts of plants

High percentages were also observed in the nematode reduction as shown in Fig. 3.11. The highest percentage (66.8) was achieved using the nematicide Carbofuran at its highest dose (2.0 g pot^{-1}), while using extracts of *Thuja orientalis* and *Thevetia neriifolia* at their highest doses achieved 56.9 and 55.1 %, respectively.

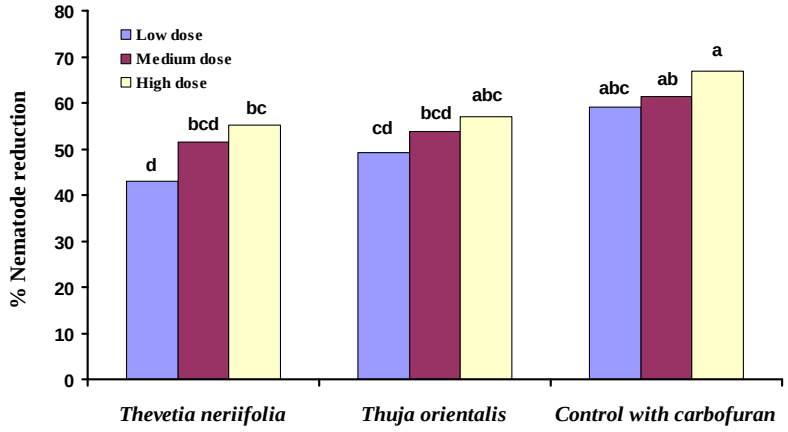


Fig. 3.11: Population reductions of *Heterodera schachtii* as influenced by hexane extracts of plants

Significant reductions in nematode build-up (Fig. 3.12) were observed at all doses following treatment with plant extracts. The highest reductions of the nematode build-up were achieved when using the highest treatment doses. The minimum values of *H. schachtii* build-up were achieved by the nematicide Carbofuran even at its lowest dose, followed by the herbal extracts of *Thuja orientalis* and *Thevetia neriifolia* at their highest

concentrations with 4.6 and 4.8 fold, respectively. No significant differences were detected between the treatments.

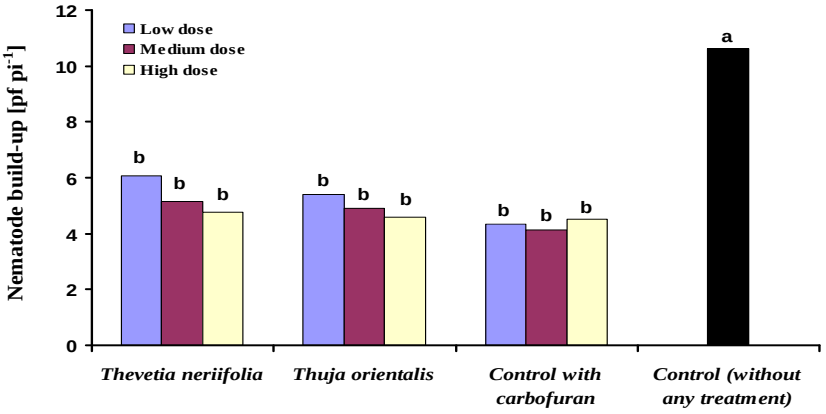


Fig. 3.12: Population build-up of *Heterodera schachtii* as influenced by hexane extracts of plants

The data on sugar beet seedlings growth response as influenced by the plant extracts using the solvent hexane are shown in Tab. 3.8. Generally, positive relations occurred between the treatments and plant growth parameters at high concentrations.

Decreases in the plant height and in the fresh and dry weights of the herbal biomass were observed at the lowest concentrations of all treatments compared to the control. The application of plant extracts at highest dosage increased the considered parameters. Based on plant height, the most effective treatment was observed to be *Thevetia neriifolia* extracts at maximal concentration, yielding a height of 16.3 cm. This was followed by Carbofuran and *Thuja orientalis* at their maximum doses with 15.5 and 14.9 cm, respectively. Furthermore, at the highest doses of the plant extracts, fresh and dry weights were also increased compared to the control. For instance, extracts of *Thevetia neriifolia* yieldeded 13.6 and 2.3 g, respectively followed by extracts of *Thuja orientalis* with 11.2 and 1.9 g, respectively while the comparable nematicide Carbofuran achieved the smallest increase even at the highest dose with 9.1 and 1.5 g, respectively.

In terms of root growth parameters, no proportional effects were observed between materials/doses and root length except for Carbofuran. The nematicide Carbofuran yielded an increase in root length with decreasing dosage. The greatest root growth as compared to the control was achieved with extracts of *Thevetia neriifolia* at a concentration of 2.5 %, resulting in 68.8 cm, while the extracts of *Thuja orientalis* recorded the greatest root length at a concentration of 1.25 % with 63 cm. On the other hand, increases in root fresh and dry

weights were observed at the highest concentrations of applied plant extracts. The greatest weight when compared to the control was recorded at the highest concentration of each plant extract. For instance, *Thevetia neriifolia* extract at a concentration of 5 % yielded 16.5 and 3.9 g in fresh and dry weight, respectively, while extracts of *Thuja orientalis* at the same concentration yielded 10.6 and 2.6 g, respectively, followed by Carbofuran at 1.0 g pot^{-1} with 7 and 1.6 g, respectively.

Tab. 3.8: Growth response of sugar beet seedlings infected with *Heterodera schachtii* as influenced by the addition of hexane extracts of plants

Plant extr.	Conc.	Shoot			Root		
		Height (cm)	Fresh wt. (g)	Dry wt. (g)	Length (cm)	Fresh wt. (g)	Dry wt. (g)
Thevetia neriifolia (%)	1.25	9^{g}	6.1^{de}	1.1^{de}	46^{ab}	6.4^{cd}	1.4^{c}
	2.5	10^{fg}	6^{de}	1.1^{de}	68.8^{a}	5.6^{cd}	1.3^{c}
	5	16.3^{b}	13.6^{b}	2.3^{ab}	62.8^{ab}	16.5^{b}	3.9^{b}
Thuja orientalis (%)	1.25	9.5^{g}	5.6^{de}	0.9^{de}	63^{ab}	5^{d}	1.2^{c}
	2.5	11.8^{efg}	8.4^{cde}	1.4^{cde}	41^{ab}	10^{cd}	2.4^{bc}
	5	14.9^{bcd}	11.2^{bc}	1.9^{bc}	43.8^{ab}	10.6^{c}	2.6^{bc}
Carbofuran (g pot^{-1})	0.5	9.4^{g}	4.6^{e}	0.8^{e}	63.3^{ab}	5.5^{cd}	1.3^{c}
	1	12.9^{cde}	7.7^{cde}	1.3^{cde}	56^{ab}	7^{cd}	1.6^{c}
	2	15.5^{bc}	9.1^{cd}	1.5^{cd}	52.5^{ab}	6.9^{cd}	1.5^{c}
Healthy plants		23.9^{a}	20.8^{a}	2.8^{a}	36.8^{b}	26.9^{a}	7.4^{a}
Control		11.9^{efg}	6.5^{de}	1.1^{de}	49^{ab}	5.4^{cd}	1.4^{c}

Plant extr. = Plant extract, Conc. = Concentration, wt = weight, Control = Plants inoculated with nematodes without any treatment, Healthy plants= Plants free of nematodes and any treatment.
The plant extracts were added to soil as three dilutions (1.25, 2.5, and 5%) of crude extract.
Mean values followed by the same letters in column are not significantly different by Tukey's test at 0.05 levels.

3.2 Enhancing the antiphytopathogenic potential of soil against the citrus nematode *Tylenchulus semipenetrans*

3.2.1 Organic and inorganic fertilisers

The effectiveness of the organic fertiliser (pigeon excrements and crushed garlic), inorganic fertiliser NH_4NO_3 and the comparable nematicide Carbofuran on reproductivity of *T. semipenetrans* and navel orange growth response were studied and evaluated.

Nematode population and fecundity were monitored. The data are presented in Tab. 3.9. The presented data reveal that all organic fertilisers as applied at three rates (25, 50 and 100 g pot^{-1}) significantly suppressed the numbers of egg masses, immature stages, final populations and eggs per egg mass, as well as the calculated nematode build-up (Pf Pi^{-1}) and egg production compared to the control. On the other hand, in the case of the inorganic fertiliser NH_4NO_3 all indications of nematode presence decreased significantly with only one exception at a level of 5 g pot^{-1}, which showed an insignificant decrease in eggs per egg mass. However, the differences in nematode suppression were noticeable between treatments and/or dosages. For instance, pigeon excrements at all levels yielded high reduction in all nematode criteria and were associated with an acute decrease in nematode build-up and egg production; next most effective was the application of crushed garlic.

No clear proportional effects were observed between doses of pigeon excrements or crushed garlic and nematode criteria. Strong reductions of nematode populations were observed. On the other hand, the inhibition of nematode counts and fecundity increased steadily by increasing the doses of NH_4NO_3.

Tab. 3.9: Reproductivity of *Tylenchulus semipenetrans* influenced by the addition of organic and inorganic fertilisers

Treat.	Dose g pot^{-1}	Nematode counts			Pf Pi^{-1}	Nematode fecundity	
		Egg masses	Immature stages	Final pop. in soil (J_2)		Eggs eggmass^{-1}	% egg prod.
Pigeon excrements	**25**	90^{cd}	186de	4824^{ef}	1.02^{efg}	76^{cde}	16.6^{ef}
	50	95^{cd}	165de	5186^{def}	1.09^{defg}	99de	22.9^{def}
	100	124^{bc}	204de	5507^{def}	1.17^{cdefg}	116^{bcd}	35.0^{cde}
Crushed garlic	**25**	78^{cd}	265^{cd}	8606^{c}	1.79^{c}	116^{bcd}	22.0^{def}
	50	76^{cd}	229de	7061^{cde}	1.47^{cde}	100^{bcde}	18.5^{ef}
	100	87^{cd}	259^{cd}	11228^{b}	2.31^{b}	99^{bcde}	20.9^{def}
NH_4NO_3	**5**	183^{ab}	447^{ab}	11218^{b}	2.37^{b}	174^{a}	77.4^{b}
	10	142^{bc}	369^{bc}	8000^{c}	1.70^{c}	152^{ab}	52.5^{c}
	20	139^{bc}	399^{b}	7332^{cd}	1.57^{cd}	129^{abc}	43.6^{cd}
Carbofuran	**0.5**	112^{c}	193de	6521^{cde}	1.37^{cdef}	96^{bcde}	26.1^{def}
	1.0	40^{d}	107^{e}	4053^{f}	0.84^{fg}	60de	5.8^{f}
	2.0	36^{d}	104^{e}	3300^{f}	0.69^{g}	59^{e}	5.2^{f}
Control		235^{a}	539^{a}	20341^{a}	4.22^{a}	175^{a}	100^{a}

Treat. = Treatment, J_2 = second stage juveniles, % Egg prod. = % Egg production, Pf Pi^{-1} = Final population / Initial population
Control = Plants inoculated with nematodes without any treatment.
Mean values followed by the same letters in column are not significantly different by Tukey's test at 0.05 levels.

The count of eggs per g root is a reliable indicator of nematode fecundity. The relationship between the concerned treatments and the count of eggs per g root is drawn in Fig. 3.13. It was observed that all fertilisers achieved a significant reduction in eggs per g root compared to the control. No proportional effects were noticeable between doses and the counts of eggs per g root when the organic fertilisers were applied. However, increasing the dose of NH4NO3 decreased the count of eggs per g root. The best reduction was achieved by using the nematicide Carbofuran at rates of 2.0 and 1.0 g pot^{-1}, resulting in 103 and 135 eggs g root^{-1}, respectively. Pigeon excrements at a dose of 25 g pot^{-1} yielded significant reduction compared to the control with 304 eggs g^{-1} root followed by crushed garlic at 50 g pot^{-1} with 425 eggs g^{-1} root.

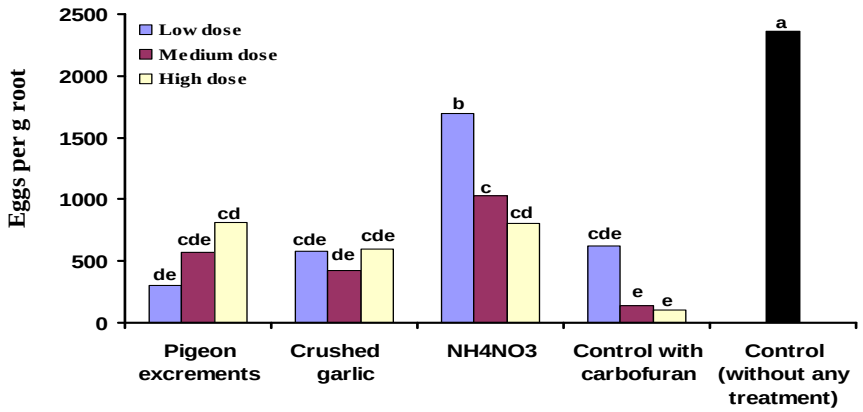


Fig. 3.13: Numbers of eggs per g root of navel orange recovered from *Tylenchulus semipenetrans* as influenced by organic and inorganic fertilisers

The percentage of nematode reduction is a reliable indicator for evaluating the ability of the treatment to reduce the nematode population in soil. The relationship between the fertilisers and the percentage of nematode reduction is presented in Fig. 3.14. The nematicide Carbofuran at rates of 1.0 and 2.0 g pot^{-1} achieved the highest percentages of nematode reduction in soil with 83.7 and 80.1%, respectively. Pigeon excrements followed Carbofuran, yielding 75.8, 74.1 and 72.4 % reduction at doses of 100, 50 and 25 g pot^{-1}, respectively. Resembling results were achieved by the application of crushed garlic and finally the inorganic fertiliser NH_4NO_3 (Fig 3.14).

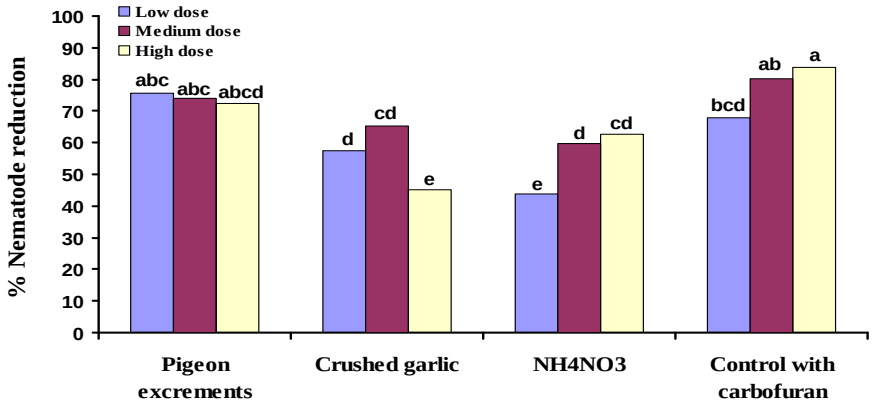


Fig. 3.14: Population reductions of *Tylenchulus semipenetrans* as influenced by organic and inorganic fertilisers

The data of navel orange seedlings growth response are listed in Tab. 3.10. The data demonstrated a positive relation between most treatments and the plant growth parameters.

Referring to the shoot growth parameters, all levels of the treatments showed increases in plant height as well as fresh and dry weight. The highest shoot measurements as compared to the control was achieved when using NH_4NO_3 at rate of 20 g pot^{-1} resulting in 59.5 cm in plant height, 52.6 g in fresh weight and 27.6 g in dry weight. Pigeon excrements and crushed garlic achieved high increases in shoot parameters especially at rates of 25 and 50 g pot^{-1}, respectively.

In the case of the root growth parameters, no clear proportional effects were observed within treatments/doses and root parameters except for treatment with NH_4NO_3, which showed a positive response in all root growth parameters. Pigeon excrements at a dose of 25 g pot-1, which yielded 29.2 cm in root length and 22.6 and 11.1 g in fresh and dry weights, respectively, achieved the highest values of root parameters as compared to the control. The next most effective treatment was NH_4NO_3 at the dose of 20 g pot^{-1}.

Tab. 3.10: Growth response of citrus seedlings infected with *Tylenchulus semipenetrans* and treated with organic and inorganic fertilisers

Treat.	Dose g pot^{-1}	Shoot			Root		
		Height (cm)	Fresh wt. (g)	Dry wt. (g)	Length (cm)	Fresh wt. (g)	Dry wt. (g)
Pigeon excrements	25	58.4^{ab}	49.3^{ab}	23.2^{bcd}	29.2^{a}	22.6^{a}	11.1^{a}
	50	50.8^{bcde}	44.6^{bc}	21.8^{cd}	25.6^{abcde}	18.5^{abcd}	8.5^{cde}
	100	46.9^{de}	37.1^{d}	21.4^{cd}	20.7^{e}	17.8^{bcde}	7.8^{e}
Crushed garlic	25	49.1^{cde}	38.9^{cd}	23.8^{abcd}	22.6^{bcde}	15.6^{de}	7.8^{e}
	50	56.6^{abc}	43.3^{bcd}	26.1^{abc}	26.4^{abcd}	18.0^{abcd}	8.5^{cde}
	100	48.5^{cde}	38.6^{cd}	22.7^{bcd}	22.6^{bcde}	14.6^{e}	7.4^{e}
NH_4NO_3	5	53.2^{abcd}	44.4^{bc}	27.7^{ab}	22.8^{bcde}	18.8^{abcd}	8.9^{bcde}
	10	55.5^{abc}	51.9^{a}	28.4^{a}	27.2^{abc}	21.1^{abc}	10.2^{abcd}
	20	59.5^{a}	52.6^{a}	27.6^{ab}	27.9^{ab}	22.2^{a}	10.7^{ab}
Carbofuran	0.5	49.7^{cde}	38.3^{cd}	24.1^{abc}	21.0^{de}	17.2^{cde}	8.5^{cde}
	1.0	50.5^{bcde}	39.3^{cd}	25.2^{abcd}	23.1^{bcde}	17.8^{bcde}	8.9^{bcde}
	2.0	53.5^{abcd}	42.4^{bcd}	26.0^{abc}	26.4^{abcd}	20.8^{abc}	10.5^{abc}
Healthy plants		51.6^{abcd}	40.6^{cd}	21.6^{cd}	26.4^{abcd}	21.4^{ab}	10.4^{abc}
Control		44.9^{e}	29.3^{e}	20.9^{d}	21.9^{cde}	17.4^{cde}	8.3^{de}

Treat. = Treatment, wt = weight, Control = Plants inoculated with nematodes without any treatment. Healthy plants= Plants free of nematodes and treatments.
Mean values followed by the same letters in column are not significantly different by Tukey's test at 0.05 levels.

3.2.2 Organic and amino acids

Three concentrations each of ascorbic acid, salicylic acid and amino acids were applied twice as foliar sprays on 1 year old navel orange seedlings infected with *Tylenchulus semipenetrans.*

The data presented in Tab. 3.11 verified that most organic and amino acids/concentrations showed strong significant effects on the egg masses, nematode final population and build-up as compared to the control. Nevertheless, the nematicide Carbofuran was more toxic to the nematodes. There was no observable correlation between amino acid concentration and nematode reproduction. Amino acids achieved the greatest reductions in nematode final populations at all concentrations. The nematode build-up ranged from 1.1 to 1.4 fold.

On the other hand, a positive relationship was observed between an increase in both organic acid concentrations and a decrease in nematode reproduction. Both organic acids showed steady reductions in nematode reproduction with increasing doses. Ascorbic acid at concentrations of 2.0 and 4.0 g L^{-1} recorded nematode build-up of 1.97 and 1.94 fold, respectively. Significant reductions were achieved in treatments of ascorbic acid at 1.0 g L^{-1} and salicylic acid at all concentrations.

Tab. 3.11: Reproductivity of *Tylenchulus semipenetrans* infecting citrus seedlings influenced by organic and amino acids as foliar spray application

Treat.	Dose per pot	Nematode counts			Pf Pi^{-1}	Nematode fecundity	
		Egg masses	Immature stages	Final pop. in soil (J_2)		Eggs $eggmass^{-1}$	% egg prod.
Ascorbic acid	1.0 g	99^{bc}	397^{abc}	10548^{bc}	2.21^{bc}	139^{abc}	33.5^{bcd}
	2.0 g	76^{bc}	354^{bc}	9423^{bc}	1.97^{bc}	110^{bcd}	20.3^{bcd}
	4.0 g	53^{bc}	263^{cd}	9361^{c}	1.94^{c}	103^{cd}	13.3^{cd}
Salicylic acid	1.0 g	121^{bc}	483^{ab}	11706^{b}	2.46^{b}	157^{ab}	46.2^{b}
	2.0 g	120^{bc}	453^{ab}	10986^{bc}	2.31^{bc}	133^{abc}	38.8^{bc}
	4.0 g	78^{bc}	371^{bc}	9780^{bc}	2.04^{bc}	118^{bc}	22.4^{bcd}
Amino acids	0.5 ml	90^{bc}	207^{def}	4966^{de}	1.05^{de}	91^{cd}	19.9^{bcd}
	1.0 ml	126^{bc}	251^{cde}	5084^{de}	1.09^{de}	117^{bc}	35.8^{bcd}
	2.0 ml	120^{bc}	296^{cd}	6600^{d}	1.40^{d}	134^{abc}	39.1^{bc}
Carbofuran	0.5 ml	112^{bc}	193^{def}	6521^{d}	1.37^{d}	96^{cd}	26.1^{bcd}
	1.0 ml	40^{bc}	107^{ef}	4053^{e}	0.84^{f}	60^{d}	5.8^{d}
	2.0 ml	36^{c}	104^{e}	3300^{e}	0.69^{f}	59^{d}	5.2^{d}
Control		235^{a}	539^{a}	20341^{a}	4.22^{a}	175^{a}	100^{a}

J_2 = second stage juveniles, Pf Pi^{-1} = Final population / Initial population. Control = Plants inoculated with nematodes without any treatment.
Mean values followed by the same letters in column are not significantly different by Tukey's test at 0.05 levels.

The count of eggs per g root was calculated and drawn in Fig. 3.15. Generally, it was observed that the concerned treatments showed wide variations in that count. All amino and organic acids achieved significant reductions in eggs per g root.

Ascorbic acid at the rate of 4.0 g pot^{-1} yielded 306 eggs g $root^{-1}$ followed by amino acids at a rate of 0.5 ml pot^{-1} and salicylic acid at a rate of 4.0 g pot^{-1}, which resulted in 387 and 443 eggs g $root^{-1}$, respectively.

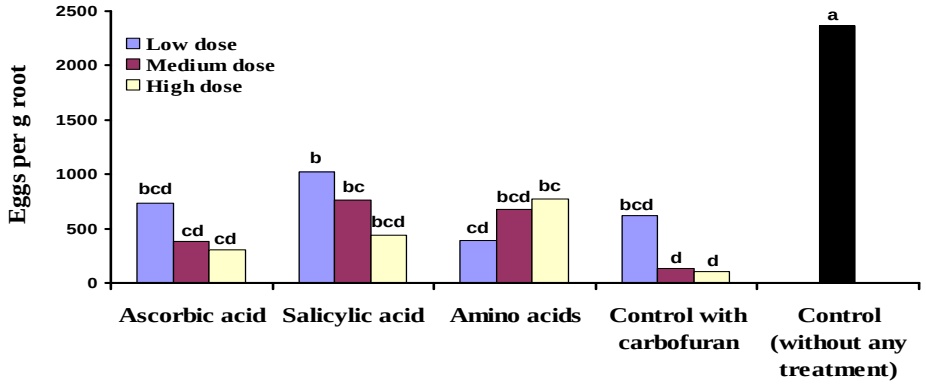


Fig. 3.15: Numbers of eggs per g root of navel orange recovered from *Tylenchulus semipenetrans* as influenced by amino and organic acids

Concerning the percentage of nematode reduction (Fig. 3.16), it was demonstrated that the effect of amino acids even at their minimum dose significantly exceeded the effect of both organic acids. The amino acids achieved the maximum nematode reduction at a rate of 0.5 ml pot^{-1} with 75.1 % followed by reductions of 74.1 and 66.8 % at the rates of 1.0 and 2.0 ml pot^{-1}, respectively. Ascorbic acid and salicylic acid followed amino acids, yielding a nematode reduction ranging from 54.2 to 41.7 %.

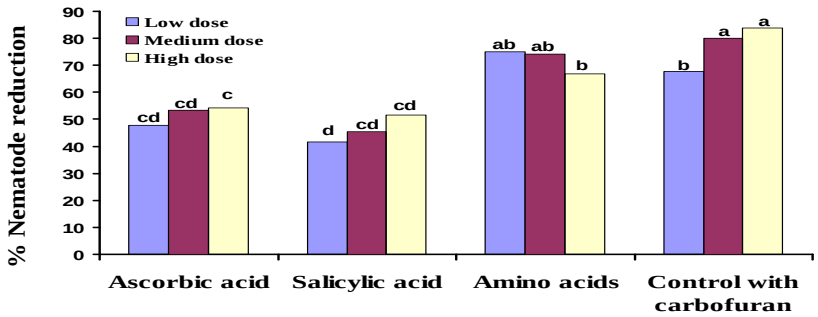


Fig. 3.16: Population reductions of *Tylenchulus semipenetrans* as influenced by amino and organic acids

Data in Tab. 3.12 showed positive relations between most of treatments and plant growth parameters.

In shoot growth parameters, all levels of the treatments exhibited increases in plant height and fresh weight when compared to the control. Amino acids at rate of 0.5 ml L^{-1} achieved the maximum percent increases with 62 cm in plant height and 49.9 g in fresh weight. Nevertheless, increasing the rate of application leads to a steady decrease in all shoot measurements. Ascorbic and salicylic acid achieved a high increase in shoot parameters at rates of 2.0 and 4.0 g L^{-1}, respectively.

In terms of root growth parameters, increases were achieved using all levels of treatments, showing nearly the same trend as previously mentioned in the shoot growth parameters. Amino acids at the rate of 0.5 ml L^{-1} yielded the maximum root length (32.3 cm) as well as the greatest fresh and dry weights with 21.8 and 13 g, respectively. Ascorbic and salicylic acids achieved high increases in root parameters at application rates of 2.0 and 4.0 g L^{-1}, respectively.

Tab. 3.12: Growth response of citrus seedlings infected with *Tylenchulus semipenetrans* as influenced by foliar spray application of organic and amino acids.

Treat.	Dose per pot	Shoot			Root		
		Height (cm)	Fresh wt. (g)	Dry wt. (g)	Length (cm)	Fresh wt. (g)	Dry wt. (g)
Ascorbic acid	1.0 g	48.9^{b}	38.4^{cd}	21.1^{abcd}	24.7^{b}	18.9^{a}	9.4^{b}
	2.0 g	54.0^{ab}	44.3^{abc}	23.4^{abcd}	27.0^{ab}	21.8^{a}	10.8^{ab}
	4.0 g	50.0^{b}	35.7^{de}	19.2^{d}	23.1^{b}	17.5^{a}	8.7^{b}
Salicylic acid	1.0 g	50.4^{b}	38.9^{cd}	20.8^{bcd}	23.6^{b}	18.6^{a}	9.6^{b}
	2.0 g	50.6^{b}	39.0^{cd}	20.4^{cd}	25.6^{ab}	20.9^{a}	9.8^{ab}
	4.0 g	51.2^{b}	41.3^{bcd}	21.6^{abcd}	26.7^{ab}	20.9^{a}	10.7^{ab}
Amino acids	0.5 ml	62.0^{a}	49.9^{a}	25.8^{ab}	32.3^{a}	21.8^{a}	13^{a}
	1.0 ml	54.1^{ab}	46.7^{ab}	21.9^{abcd}	27.1^{ab}	21.8^{a}	10.6^{ab}
	2.0 ml	50.2^{b}	41.3^{bcd}	19.5^{d}	25.7^{ab}	20.8^{a}	10^{ab}
Carbofuran	0.5 ml	49.7^{b}	38.3^{cd}	24.1^{abcd}	21.0^{b}	17.2^{a}	8.5^{b}
	1.0 ml	50.5^{b}	39.3^{cd}	25.2^{abc}	23.1^{b}	17.8^{a}	8.9^{b}
	2.0 ml	53.5^{ab}	42.4^{bcd}	26.0^{a}	26.4^{ab}	20.8^{a}	10.5^{ab}
Healthy plants		51.6^{b}	40.6^{bcd}	21.6^{abcd}	26.4^{ab}	21.4^{a}	10.4^{ab}
Control		44.9^{b}	29.3^{e}	20.9^{bcd}	21.9^{b}	17.4^{a}	8.3^{b}

Treat. = Treatment, wt = weight, Control = Plants inoculated with nematodes without any treatment, Healthy plants= plants free of nematodes and treatments.
Mean values followed by the same letters in column are not significantly different by Tukey's test at 0.05 levels.

3.2.3 Microbial agents

Dilutions of *Dactylella brochopaga*, *Nematoctonus concurrence* and *Bacillus thuringiensis* as a soil drench, were each applied at three different levels to test their ability

against the target nematode pest *Tylenchulus semipenetrans* in citrus seedlings as well as their effect on plant growth response under greenhouse conditions.

The data obtained after four months of co-existence of each microorganism with the nematode (Tab. 3.13) illustrated that the microbial agents clearly impaired nematode reproductivity. All treatments exhibited significant decreases in number of adult and pre-adult females and nematode final population in soil, and consequently in the calculated nematode build-up. Furthermore, a strong reduction in the counts of egg masses, eggs per egg masses, eggs per g root and % egg production were observed. Differences in nematode suppression were noticeable among treatments and/or doses. The inhibitions of the nematode counts and fecundity were increased steadily by increasing the filtrate concentration except in the case of *Bacillus thuringiensis*, which showed no clear proportional effects between concentrations and nematode reduction.

The strongest reduction in the nematode parameters was achieved using filtrates of the antagonistic fungus *Nematoctonus concurrence* at a concentration level of 100 % that had inimical effects on all nematode parameters. Furthermore, this treatment was more effective than the nematicide Carbofuran even at its highest dose.

Dactylella brochopaga has also yielded strong reductions in all nematode criteria and the nematode build-up especially at high concentrations. This was followed by *Bacillus thuringiensis*, which yielded significant reductions in all nematode parameters when applied at 2.0 g pot^{-1}.

Tab. 3.13: Reproductivity of *Tylenchulus semipenetrans* infecting citrus seedlings as influenced by microbial bio-agents

Treat.	Conc.	Nematode counts			Pf Pi^{-1}	Nematode fecundity	
		Egg masses	Immature stages	Final pop. in soil (J_2)		Eggs $eggmass^{-1}$	% egg prod.
Dactylella brochopaga	**50 %**	160^{b}	401^{b}	7818^{b}	1.68^{b}	156^{ab}	60.7^{b}
	75 %	97^{bcde}	290^{bc}	5833^{bcde}	1.24^{bcd}	132^{ab}	31.1^{cd}
	100 %	33^{ef}	86^{fg}	3910^{ef}	0.81^{de}	109^{abc}	8.7^{cd}
Nematoctonus concurrence	**50 %**	100^{bcde}	228^{cde}	7058^{bc}	1.48^{bc}	117^{abc}	28.4^{cd}
	75 %	50^{cdef}	142^{defg}	4981^{def}	1.03^{cde}	98^{bc}	11.9^{cd}
	100 %	23^{f}	70^{g}	3393^{f}	0.70^{e}	58^{c}	3.2^{d}
Bacillus thuringiensis [g pot^{-1}]	**1.0**	77^{cdef}	180^{cdefg}	5126^{cdef}	1.07^{cde}	90^{bc}	16.9^{cd}
	2.0	43^{def}	123^{efg}	3726^{f}	0.78^{e}	60^{c}	6.3^{cd}
	4.0	117^{bc}	243^{cd}	7332^{b}	1.54^{b}	118^{abc}	33.6^{c}
Carbofuran	**0.5**	112^{bcd}	193^{cdef}	6521^{bcd}	1.37^{bc}	96^{bc}	26.1^{cd}
	1.0	40^{def}	107^{fg}	4053^{ef}	0.84^{de}	60^{c}	5.8^{d}
	2.0	36^{ef}	104^{fg}	3300^{f}	0.69^{e}	59^{c}	5.2^{d}
Control		235^{a}	539^{a}	20341^{a}	4.22^{a}	175^{a}	100^{a}

Treat. = Treatment, J_2 = second stage juveniles, % Egg prod. = % Egg production, Pf Pi^{-1} = Final population / Initial population
Conc. = Concentration, Control = Plants inoculated with nematodes without any treatment.
Mean values followed by the same letters in column are not significantly different by Tukey's test at 0.05 levels.

Significant reductions using all microbial agents were achieved in the count of eggs per g root as illustrated in Fig. 3.17. The strongest reduction was achieved using the fungus *N. concurrence* at a concentration of 100 %, resulting in 73 eggs g $root^{-1}$. The nematicide Carbofuran at its highest application rate yielded 103 eggs g $root^{-1}$, followed by *Bacillus thuringiensis* at 2.0 g pot^{-1}, which yielded 120 eggs g $root^{-1}$. *D. brochopaga* achieved the highest effectiveness in reduction at its highest concentration, with 191 eggs g $root^{-1}$.

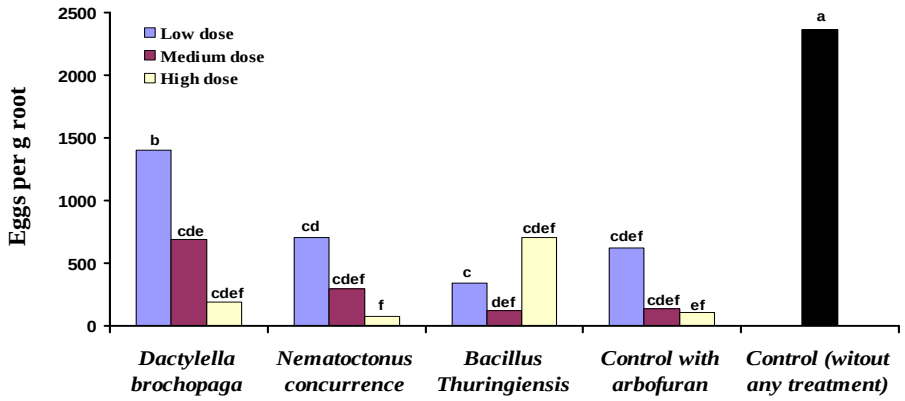


Fig. 3.17: Numbers of eggs per g root recovered from *Tylenchulus semipenetrans* as influenced by microbial agents

High percentages were observed in nematode reduction using all microbial agents, ranging from 60.3 % with *D. brochopaga* at 50 % filtrate concentration to 83.5 % with *N. concurrence* at its highest concentration. No significant differences were noticed between Carbofuran and *Bacillus thuringiensis* or *N. concurrence* at their highest rates of application which yielded nematode reductions of 83.7, 81.6 and 83.5 %, respectively.

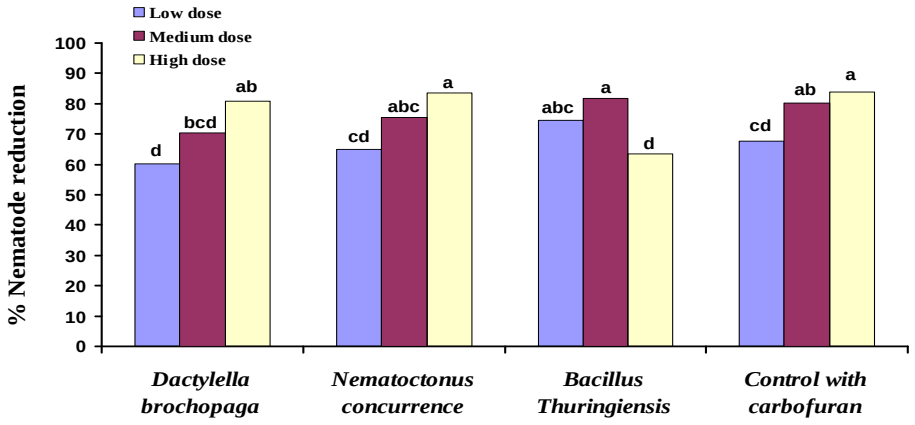


Fig. 3.18: Population reductions of *Tylenchulus semipenetrans* as influenced by microbial agents

The data in Tab. 3.14 indicated that reductions in the nematode populations as a result of the application of microbial treatments supported the vegetative improvement.

With respect to the shoot growth parameters, most levels of application achieved positive increases when compared to the control especially in terms of shoot height and fresh weight. This increase was more evident with *Bacillus thuringiensis* at a rate of 2.0 g pot^{-1}, which yielded 59.6 cm in plant height and 49.5 and 27.4 g in fresh and dry weights, respectively, followed by *B. thuringiensis* at the rate of 1.0 g pot^{-1}. The filtrates of

antagonistic fungi *D. brochopaga* and *N. concurrence* at their highest concentrations also achieved a high increase in all shoot parameters. For instance, the filtrates of *N. concurrence* at a rate of 100 % yielded 53.4 cm in plant height and 41.1 and 21.5 g in fresh and dry weights, respectively, this result was followed by filtrate of *D. brochopaga* at the same concentration.

According to the root growth parameters, the biological agents achieved nearly the same trend as previously mentioned in the shoot growth parameters. *Bacillus thuringiensis* at a rate of 2.0 g pot^{-1} yielded the maximum measurements with 28.6 cm in root length and 21.5 and 10.5 g in fresh and dry weights, respectively. The filtrates of the antagonistic fungi *D. brochopaga* and *N. concurrence* at their highest concentrations also achieved high increases in all root parameters.

Tab. 3.14: Growth response of citrus seedlings infected with *Tylenchulus semipenetrans* treated with microbial bio-agents

Treat.	Conc.	Shoot			Root		
		Height (cm)	Fresh wt. (g)	Dry wt. (g)	Length (cm)	Fresh wt. (g)	Dry wt. (g)
Dactylella brochopaga	50 %	49.0^{cd}	37.4^{c}	19.5^{f}	24.3^{abcd}	17.8^{abcd}	8.2^{b}
	75 %	51.3^{bc}	38.4^{bc}	20.7^{ef}	25.5^{abcd}	18.4^{abcd}	8.6^{ab}
	100 %	52.3^{bc}	39.7^{bc}	21.4^{cdef}	26.4^{ab}	19.0^{abcd}	9.3^{ab}
Nematoctonus concurrence	50 %	48.9^{cd}	39.5^{bc}	21.1^{def}	24.1^{abcd}	16.6^{d}	8.0^{b}
	75 %	50.2^{bcd}	39.3^{bc}	20.6^{ef}	24.9^{abcd}	16.7^{d}	7.7^{b}
	100 %	53.4^{abc}	41.1^{bc}	21.5^{cdef}	27.7^{a}	18.7^{abcd}	8.6^{ab}
Bacillus thuringiensis [g pot^{-1}]	1.0	56.3^{ab}	43.7^{ab}	25.4^{abc}	26.1^{abc}	20.6^{abc}	9.4^{ab}
	2.0	59.6^{a}	49.5^{a}	27.4^{a}	28.6^{a}	21.5^{a}	10.5^{a}
	4.0	50.8^{bcd}	39.6^{bc}	22.0^{bcdef}	22.3^{bcd}	19.5^{abcd}	8.9^{ab}
Carbofuran	0.5	49.7^{cd}	38.3^{bc}	24.1^{abcde}	21.0^{d}	17.2^{cd}	8.5^{ab}
	1.0	50.5^{bcd}	39.3^{bc}	25.2^{abcd}	23.1^{bcd}	17.8^{bcd}	8.9^{ab}
	2.0	53.5^{abc}	42.4^{bc}	26.0^{ab}	26.4^{ab}	20.8^{abc}	10.5^{a}
Healthy plants		51.6^{bc}	40.6^{bc}	21.6^{cdef}	26.4^{ab}	21.4^{ab}	10.4^{a}
Control		44.9^{d}	29.3^{d}	20.9^{ef}	21.9^{cd}	17.4^{cd}	8.3^{ab}

Treat. = Treatment, Conc. = Concentration, wt =weight, Control = Plants inoculated with nematodes without any treatment, Healthy plants= plants free of nematodes and treatments.
Mean values followed by the same letters in column are not significantly different by Tukey's test at 0.05 levels.

3.2.4 Plant extracts

Plant extract dilutions of *Thevetia neriifolia* and *Thuja orientalis* extracted with ethyl acetate or hexane and treated as a soil drench were used at four different levels to test their effectiveness against the target nematode pest *Tylenchulus semipenetrans* in citrus seedlings under greenhouse conditions.

The data presented in Tab. 3.15 indicated that plant extracts of *Thevetia neriifolia* and *Thuja orientalis* using the solvent ethyl acetate clearly impaired nematode reproductivity. All treatments exhibited significant reductions of egg masses, immature stages, nematode final population in the soil and consequently the nematode build-up. Furthermore, high reductions in the counts of eggs per egg masses, eggs per g root and percentage of egg production were observed. Differences in nematode suppression were

noticeable among treatments and/or doses. In both plant extracts the inhibitions of nematode counts and fecundity were increased steadily by increasing the extract concentration to a value of 5 % then decreased again with a concentration of 10 %. *Thuja orientalis* achieved greater reduction in nematode parameters at all concentration levels when compared to *Thevetia neriifolia*.

A concentration of 5 % was the optimal value for reducing nematode counts and fecundity for both extracts. At this concentration, *Thuja orientalis* and *Thevetia neriifolia* yielded their highest reductions in nematode criteria. For instance, nematode build-up in the two extracts was determined to be 1.4 and 1.7 fold, respectively.

Tab. 3.15: Reproductivity of *Tylenchulus semipenetrans* infecting citrus seedlings as influenced by the addition of botanical extracts produced using ethyl acetate

Plant extract	Conc.	Nematode counts			Pf Pi^{-1}	Nematode fecundity	
		Egg masses	Immature stages	Final pop. in soil (J_2)		Eggs $eggmass^{-1}$	% egg prod.
Thevetia neriifolia	**1.25%**	137^{bc}	422^{b}	11526^{b}	2.42^{b}	157^{ab}	52.3^{b}
	2.25%	120^{bc}	395^{b}	10355^{bcd}	2.17^{bc}	144^{abc}	42.0^{bcd}
	5%	94^{bcd}	272^{cd}	8297^{de}	1.73^{cd}	119^{bcd}	27.2^{cdef}
	10%	124^{bc}	326^{bc}	9229^{cd}	1.9^{bc}	132^{abcd}	39.8^{bcd}
Thuja orientalis	**1.25%**	141^{b}	417^{b}	11280^{bc}	2.37^{b}	137^{abc}	47^{bc}
	2.25%	134^{bc}	325^{bc}	9717^{bcd}	2.04^{bc}	105^{bcde}	34.2^{bcde}
	5%	83^{cd}	167^{de}	6532^{e}	1.36^{d}	80^{de}	16.1^{ef}
	10%	96^{bc}	166^{de}	8888^{d}	1.83^{cd}	93^{cde}	21.7^{def}
Carbofuran	**0.5**	112^{bc}	193^{de}	6521^{e}	1.37^{d}	96^{cde}	26.1^{cdef}
	1.0	40^{d}	107^{e}	4053^{f}	0.84^{e}	60^{e}	5.8^{f}
	2.0	36^{d}	104^{e}	3300^{f}	0.69^{e}	59^{e}	5.2^{f}
Control		235^{a}	539^{a}	20341^{a}	4.22^{a}	175^{a}	100^{a}

Conc. = Concentration, J_2 = second stage juveniles, % Egg prod. = % Egg production, Pf Pi^{-1} = Final population / Initial population
Control = Plants inoculated with nematodes without any treatment.
Mean values followed by the same letters in column are not significantly different by Tukey's test at 0.05 levels.

Significant reductions in eggs per g root were observed using both plant extracts as illustrated in Fig. 3.19. The highest reduction using the extracts of *Thuja orientalis* was achieved at a concentration of 5 % with 300 eggs g $root^{-1}$, followed by a concentration of 10 % which yielded 419 eggs g $root^{-1}$. *Thevetia neriifolia* was most effective at a

concentration of 5 % with 536 eggs g root^{-1}. The nematicide Carbofuran at its highest application rate yielded 103 eggs g root^{-1}.

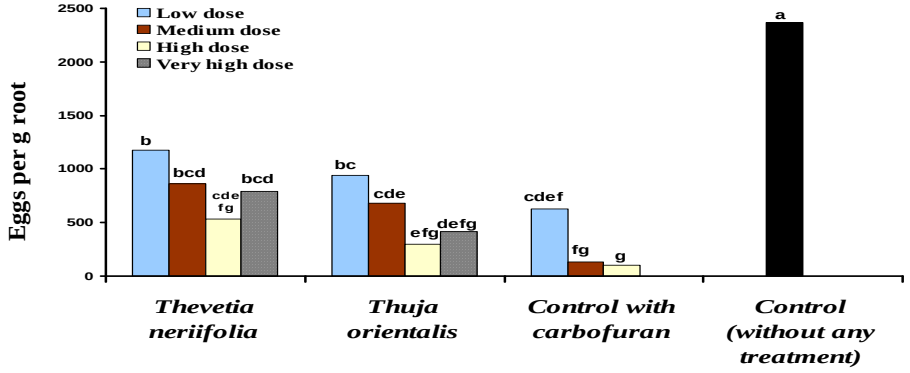


Fig. 3.19: Numbers of eggs per g root recovered from *Tylenchulus semipenetrans* as influenced by ethyl acetate extracts of plants

In terms of the percentage of nematode reduction (Fig. 3.20), it was shown that extracts of *Thuja orientalis* and *Thevetia neriifolia* at all doses achieved significantly high reductions in nematode population in the soil. *Thuja orientalis* and *Thevetia neriifolia* achieved their maximum nematode reductions at a concentration of 5 % resulting in a reduction of 67.9 and 59 %, respectively. The extracts of *Thuja orientalis* were more effective than the *Thevetia neriifolia*.

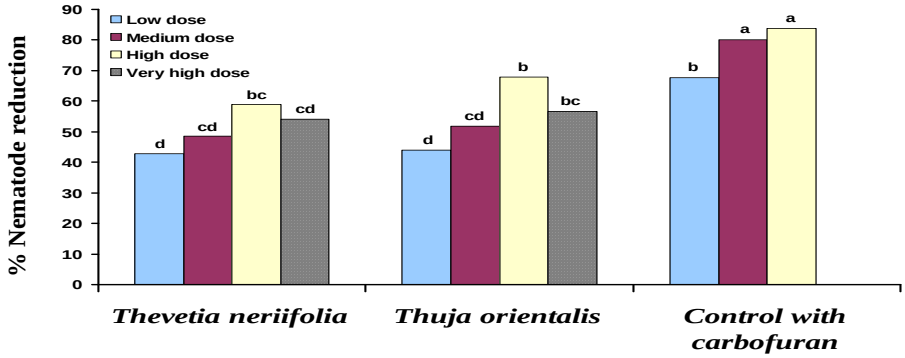


Fig. 3.20: Population reductions of *Tylenchulus semipenetrans* as influenced by ethyl acetate extracts of plants

The data in Tab. 3.16 showed vegetative improvement of citrus seedlings as a result of applying the plant extracts extracted by ethyl acetate.

In terms of shoot parameters, most treatment levels yielded increases when compared to control values, especially in shoot height and fresh weight. These increases were more recognisable with the plant extracts of *Thuja orientalis* at concentration of 5 %, which yielded the highest values at 55.3 cm height and 43.5 and 22.7 g in fresh and dry

weights, respectively. The extracts of *Thevetia neriifolia* at concentration of 10 % also achieved increases in all shoot parameters, yielding 51.1 cm height and 39.7 and 22.1 g fresh and dry weights, respectively.

As concerns the root parameters, positive increments were achieved using some treatments, primarily at a concentration rate of 5 % for both extracts. *Thuja orientalis* at a rate of 5 % yielded 26.1 cm in root length and 22.3 and 10.7 g in fresh and dry weights, respectively. The extract of *Thevetia neriifolia* also achieved its highest rate in all root parameters at concentration of 5 %, with 25.3 cm in root length and 20.9 and 10.1 g in fresh and dry weights, respectively.

Tab. 3.16: Growth response of citrus seedlings infected with *Tylenchulus semipenetrans* as influenced by the addition of botanical extracts produced using ethyl acetate

Plant extract	Conc.	Shoot			Root		
		Height (cm)	Fresh wt. (g)	Dry wt. (g)	Length (cm)	Fresh wt. (g)	Dry wt. (g)
Thevetia neriifolia	1.25%	46.5^{bc}	32.9^{bc}	19.3^{c}	21.7^{a}	18.3^{ab}	8.8^{a}
	2.25%	48.6^{abc}	37.1^{ab}	21.7^{abc}	24.5^{a}	20.0^{ab}	9.5^{a}
	5%	50.5^{abc}	40.2^{a}	20.9^{bc}	25.3^{a}	20.9^{ab}	10.1^{a}
	10%	51.1^{abc}	39.7^{a}	22.1^{abc}	24.5^{a}	20.7^{ab}	9.3^{a}
Thuja orientalis	1.25%	47.6^{bc}	38.9^{ab}	21.0^{bc}	24.1^{a}	19.4^{ab}	9.0^{a}
	2.25%	52.1^{abc}	41.8^{a}	22.4^{abc}	25.2^{a}	20.7^{ab}	9.5^{a}
	5%	55.3^{a}	43.5^{a}	22.7^{abc}	26.1^{a}	22.3^{a}	10.7^{a}
	10%	52.6^{abc}	41.7^{a}	22.3^{abc}	25.2^{a}	21.4^{ab}	9.8^{a}
Carbofuran	0.5	49.7^{abc}	38.3^{ab}	24.1^{ab}	21.0^{a}	17.2^{b}	8.5^{a}
	1.0	50.5^{abc}	39.3^{ab}	25.2^{ab}	23.1^{a}	17.8^{b}	8.9^{a}
	2.0	53.5^{ab}	42.4^{a}	26.0^{a}	26.4^{a}	20.8^{ab}	10.5^{a}
Healthy plants		51.6^{abc}	40.6^{a}	21.6^{abc}	26.4^{a}	21.4^{ab}	10.4^{a}
Control		44.9^{c}	29.3^{c}	20.9^{bc}	21.9^{a}	17.4^{b}	8.3^{a}

Conc. = Concentration, Control = Plants inoculated with nematodes without any treatment, wt =weight,
Healthy plants= Plants free of nematodes and any treatment.
Mean values followed by the same letters in column are not significantly different by Tukey's test at 0.05 levels.

The data presented in Tab. 3.17 indicated that the hexane extracts of *Thuja orientalis* and *Thevetia neriifolia* strongly impaired the nematode reproductivity. All

treatments exhibited significant reductions in counts of egg masses, immature stages, nematode final population in soil and consequently nematode build-up. Reductions in the counts of eggs per egg mass and percentage of egg production were observed. Differences in nematode suppression were noticeable among treatments and/or doses. In both plant extracts, inhibition of nematode counts and fecundity increased steadily by increasing the extract concentration up to a concentration of 5 %, and then decreased again with a concentration of 10 %. At all concentration levels, *Thuja orientalis* was more effective in reducing nematode parameters than *Thevetia neriifolia*.

The concentration of 5 % was the optimal for reducing both nematode counts and fecundity for both extracts. At this concentration, *Thuja orientalis* and *Thevetia neriifolia* were most effective in reducing nematode criteria. For instance, they achieved a nematode build-up of 1.7 and 2.0 fold, respectively.

Tab. 3.17: Reproductivity of *Tylenchulus semipenetrans* infecting citrus seedlings as influenced by the addition of botanical extracts produced using hexane

Plant extract	Conc.	Nematode counts			Pf Pi^{-1}	Nematode fecundity	
		Egg masses	Immature stages	Final pop. in soil (J_2)		Eggs $eggmass^{-1}$	% egg prod.
Thevetia neriifolia	1.25%	133^{b}	416^{ab}	12287^{b}	2.57^{b}	148^{abc}	47.9^{b}
	2.25%	118^{b}	404^{bc}	11164^{bc}	2.34^{bc}	160^{ab}	45.9^{b}
	5%	101^{b}	327^{bcd}	9674^{cde}	2.02^{cd}	134^{abcd}	32.9^{bc}
	10%	134^{b}	367^{bc}	10429^{bcd}	2.19^{bc}	133^{abcd}	43.3^{bc}
Thuja orientalis	1.25%	121^{b}	420^{ab}	12433^{b}	2.59^{b}	139^{abcd}	40.9^{bc}
	2.25%	124^{b}	406^{bc}	10106^{bcde}	2.13^{bcd}	116^{bcd}	35.0^{bc}
	5%	102^{b}	240^{de}	7992^{ef}	1.67^{de}	105^{cde}	26.0^{cd}
	10%	117^{b}	290^{cde}	8316^{def}	1.74^{de}	111^{bcd}	31.6^{bc}
Carbofuran	0.5	112^{b}	193^{ef}	6521^{f}	1.37^{e}	96^{de}	26.1^{cd}
	1.0	40^{c}	107^{f}	4053^{g}	0.84^{f}	60^{e}	5.8^{d}
	2.0	36^{c}	104^{f}	3300^{g}	0.69^{f}	59^{e}	5.2^{d}
Control		235^{a}	539^{a}	20341^{a}	4.22^{a}	175^{a}	100^{a}

Conc. = Concentration, J_2 = second stage juveniles, % Egg prod. = % Egg production, Pf Pi^{-1} = Final population / Initial population
Control = Plants inoculated with nematodes without any treatment.
Mean values followed by the same letters in column are not significantly different by Tukey's test at 0.05 levels.

Significant reductions in the counts of eggs per g root were observed by using both plant extracts as illustrated in Fig. 3.21. The highest reduction by using the extracts of *Thuja orientalis* was achieved at a concentration of 5 % with 525 eggs g root^{-1}, followed by a concentration of 10 % which yielded 583 eggs g root^{-1}. *Thevetia neriifolia* achieved the highest reduction at a concentration of 5 % with 670 eggs g root^{-1}. The nematicide Carbofuran at its highest application rate yielded 103 eggs g root^{-1}

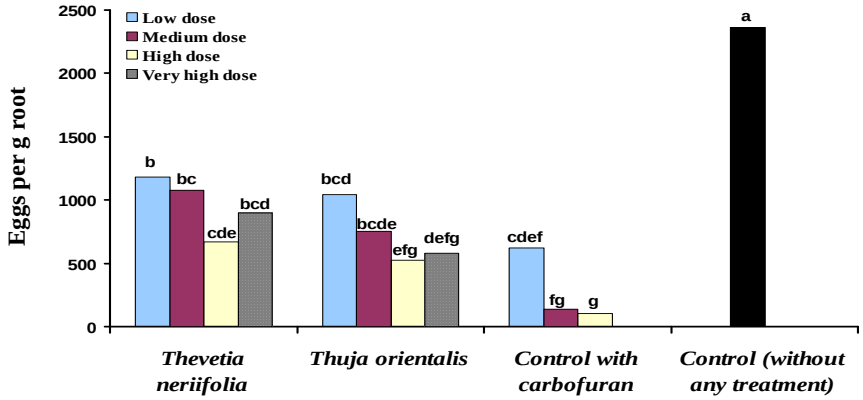


Fig. 3.21: Numbers of eggs per g root recovered from *Tylenchulus semipenetrans* as influenced by hexane extracts of plants

According to the percentage of nematode reduction (Fig. 3.22), it can be concluded that the extracts of both plant extracts at all applied doses achieved significant reductions in nematode population in the soil. *Thuja orientalis* and *Thevetia neriifolia* achieved maximum nematode reductions at a concentration of 5 %, which resulted in 60.5 and 52.2 % reduction, respectively. The extracts of *Thuja orientalis* were more effective than those of *Thevetia neriifolia*.

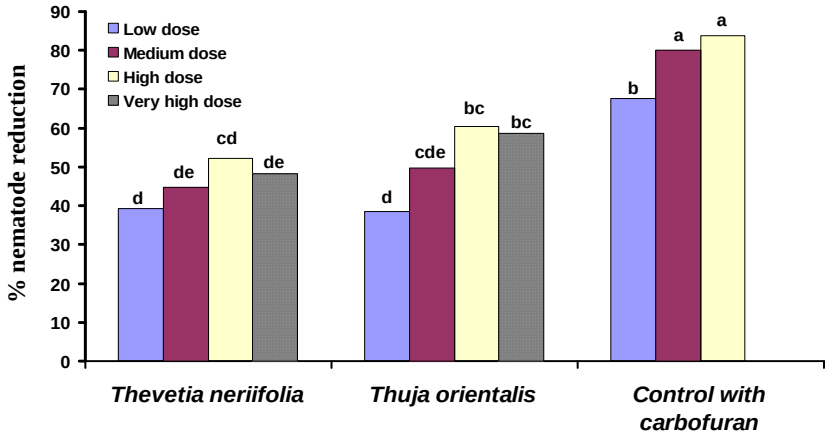


Fig. 3.22: Population reductions of *Tylenchulus semipenetrans* as influenced by hexane extracts of plants.

The data in Tab. 3.18 reveal an increase in plant measurements. This increase was achieved due to the reduction of nematode population as a result of applying the hexane plant extracts.

In the case of shoot parameters, most treatments yielded increases in shoot height and fresh weight when compared to the control. This increase was more evident with plant extracts of *Thuja orientalis* at a concentration of 5 % which yielded 51.6 cm height and 41.3 and 21.1 g in fresh and dry weights, respectively, followed by a concentration of 10 %. The same trend was observed with the extracts of *Thevetia neriifolia* at a concentration of 5 %, these also yielded increases in all shoot parameters, namely 49.1 cm height and 38.9 and 21.1 g in fresh and dry weights, respectively.

Referring to the root measurements, the greatest increases were achieved using both extracts of *Thuja orientalis* and *Thevetia neriifolia* at rates of 10 and 5 %, respectively. *Thuja orientalis* at rate of 10 % yielded 25.4 cm length and 22.3 and 10.7 g fresh and dry weights, respectively. On the other hand, the extract of *Thevetia neriifolia* yielded the greatest value in all root measurements at a concentration of 5 % with 25.3 cm length and 20.1 and 9.6 g in fresh and dry weights, respectively.

Tab. 3.18: Growth response of citrus seedlings infected with *Tylenchulus semipenetrans* as influenced by the addition of botanical extracts produced using hexane.

Plant extract	Conc.	Shoot			Root		
		Height (cm)	Fresh wt. (g)	Dry wt. (g)	Length (cm)	Fresh wt. (g)	Dry wt. (g)
Thevetia neriifolia	1.25%	45.9^{bc}	30.1^{de}	18.1^{e}	20.7^{bc}	16.6^{de}	8.3^{b}
	2.25%	46.9^{bc}	32.7^{bcde}	19.0^{de}	21.4^{abc}	17.6^{bcde}	8.4^{ab}
	5%	49.1^{abc}	38.9^{ab}	21.1^{abcde}	25.3^{abc}	20.1^{abcde}	9.6^{ab}
	10%	48.9^{abc}	36.9^{abcd}	20.6^{cde}	23.8^{abc}	19.9^{abcde}	9.3^{ab}
Thuja orientalis	1.25%	46.9^{bc}	31.2^{cde}	25.3^{abc}	20.5^{c}	16.2^{e}	8.3^{b}
	2.25%	50.4^{abc}	39.9^{ab}	25.6^{ab}	21.2^{abc}	19.1^{abcde}	8.9^{ab}
	5%	51.6^{ab}	41.3^{a}	21.1^{abcde}	26.6^{a}	20.5^{abcd}	9.5^{ab}
	10%	51.1^{ab}	40.9^{a}	23.4^{abcd}	25.4^{abc}	22.3^{a}	10.7^{a}
Carbofuran	0.5	49.7^{abc}	38.3^{abc}	24.1^{abc}	21.0^{abc}	17.2^{cde}	8.5^{ab}
	1.0	50.5^{abc}	39.3^{ab}	25.2^{abc}	23.1^{abc}	17.8^{bcde}	8.9^{ab}
	2.0	53.5^{a}	42.4^{a}	26.0^{a}	26.4^{a}	20.8^{abc}	10.5^{ab}
Healthy plants		51.6^{ab}	40.6^{a}	21.6^{abcde}	26.4^{ab}	21.4^{ab}	10.4^{ab}
Control		44.9^{c}	29.3^{e}	20.9^{bcde}	21.9^{abc}	17.4^{bcde}	8.3^{b}

Conc. = Concentration, J_2 = second stage juveniles, Control = Plants inoculated with nematodes without any treatment.
Healthy plants= Plants free of nematodes and any treatment.
Mean values followed by the same letters in column are not significantly different by Tukey's test at 0.05 levels.

3.3 Application of different rootstocks as a mean to control citrus nematode

3.3.1 Relative susceptibility of citrus rootstocks

Penetration, development and potential reproduction of *Tylenchulus semipenetrans* were estimated on some selected rootstocks. Plant growth response was observed and recorded. Sour orange *Citrus aurantium* was used as a comparable standard host. Nematode counts, reproduction and plant growth response data are presented in Tab. 3.19, 3.20 and Fig. 3.23, 3.24 and 3.25. It could be generally concluded that nematode penetration, number of individuals reaching adulthood, fecundity, and consequently the nematode reproduction were varied from one rootstock to another.

The rates of nematode penetration as presented in Fig. 3.23 showed wide variations compared to Sour orange (6.5 %). Rough lemon and Cleopatra mandarin demonstrated more susceptibility with penetration rates of 13.6 and 8.2 %, respectively. Subsequently, the nematode numbers as well as female fecundity (count of eggs per egg mass and eggs per g root) in Tab. 3.19 recorded high rates. Non-significant differences were observed between Sour orange and Cleopatra mandarin in all nematode counts and the count of eggs per g root, while the rootstock Rough lemon achieved a high level of susceptibility and yielded significant differences in both nematode counts and fecundity as compared to all concerned rootstocks.

Rangpur lime, Volkamer lemon and Japanese bitter orange were more resistant with penetration rates of 4.5, 1.9 and 1.0 %, respectively. Subsequently, the nematode counts as well as female fecundity (eggs per egg mass and eggs per g root) were lower. Non-significant differences were noticed between sour orange and Rangpur lime. Japanese bitter orange and Volkamer lemon achieved significantly high resistance rates in both nematode count and fecundity compared to all other rootstocks used.

Japanese bitter orange yielded the best rate of resistant against the citrus nematode *T. semipenetrans* among all tested rootstocks.

Tab. 3.19: Reproductivity of *Tylenchulus semipenetrans* as influenced by using different citrus rootstocks.

Citrus rootstock	Nematode counts				Nematode fecundity		P.R.I.
	(J_2) in soil	DS	EM	Final pop.	Eggs EM^{-1}	% egg prod.	
Cleopatra mandarin	13864^{b}	247^{b}	161^{b}	14272^{b}	144^{b}	47.7^{b}	57.6^{b}
Japanese bitter orange	1244^{c}	48^{d}	12^{d}	1304^{c}	36^{e}	0.9^{e}	5.3^{c}
Rangpur lime	12604^{b}	133^{cd}	93^{cd}	12828^{b}	119^{bc}	22.7^{cd}	51.7^{b}
Rough lemon	24110^{a}	402^{a}	278^{a}	24790^{a}	175^{a}	100^{a}	100^{a}
Volkamer lemon	2870^{c}	69^{d}	27^{d}	2966^{c}	44^{d}	2.4^{e}	12.0^{c}
Sour orange	11463^{b}	200^{bc}	125^{b}	11788^{b}	118^{c}	30.3^{bc}	47.6^{b}

J_2 = Second stage juvenile, DS = Developmental stages, EM = Egg masses, Final pop. = Final population (sum of J_2+ DS+ EM)
PRI = Potential reproductive index
Mean values followed by the same letters in column are not significantly different by Tukey's test at 0.05 levels.

Referring to Fig. 3.23, 3.24 and 3.25, it can be observed that the rootstocks used showed wide variation in the rate of penetration, nematode build-up (Pf Pi^{-1}) and eggs per g root. From these relations it has been concluded that these rootstocks can be divided into three groups according to their ability to regulate the citrus nematode *Tylenchulus. semipenetrans* as follows:

1. Highly sensitive — Rough lemon.
2. Moderately resistant — Cleopatra mandarin, Rangpur lime and Sour orange.
3. Highly resistant — Japanese bitter orange and Volkamer lemon.

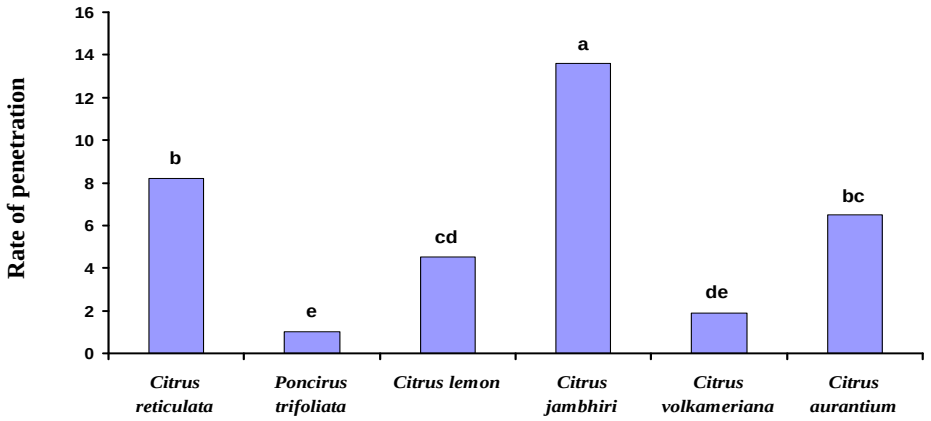


Fig. 3.23: Rate of penetration of *Tylenchulus semipenetrans* on different citrus rootstocks

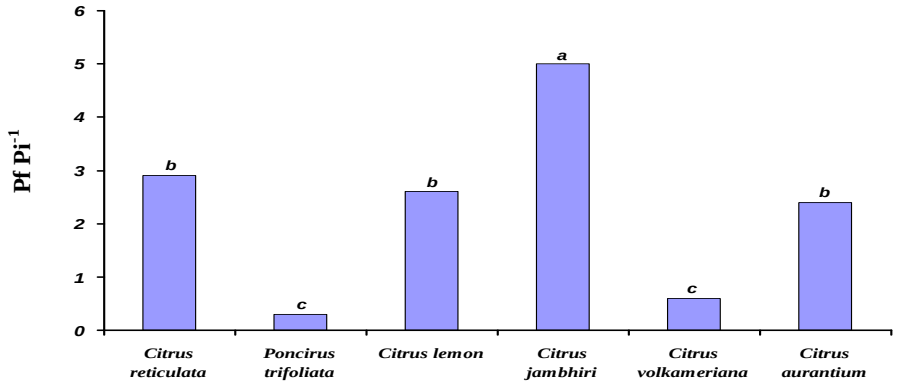


Fig. 3.24: Population build-up of *Tylenchulus semipenetrans* on different citrus rootstocks

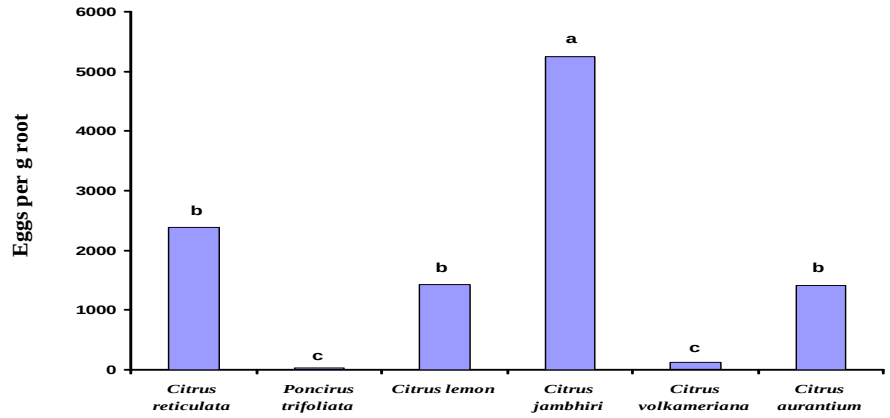


Fig. 3.25: Numbers of eggs per g root of *Tylenchulus semipenetrans* on different citrus rootstocks

The shoot and root fresh weights of citrus rootstocks as influenced by introduction of citrus nematodes are listed in Tab. 3.20. The data presents clear relationships between inoculation and plant growth measurements in each rootstock. All inoculated rootstocks showed reductions in plant measurements, as compared to rootstocks free of nematodes (control), except for shoot fresh weight of Rough lemon, which showed no differences. The reduction rates of shoot fresh weight ranged from 0 % with Rough lemon to 11.3 % with Rangpur lime. The reduction rates of root fresh weight ranged from 1.7 % with Japanese bitter orange to 20.4 % with Volkamer lemon.

Tab. 3.20: Growth response of citrus rootstocks as influenced by the infection of the citrus nematode *Tylenchulus semipenetrans*

Citrus rootstock	Shoot fresh weight (g)			Root fresh weight (g)		
	Inoc.	Check	% red.	Inoc.	Check	% red.
Cleopatra mandarin	30.8	32.8	6.1	9.7	10.6	8.5
Japanese bitter orange	36.3	36.7	1.1	11.9	12.1	1.7
Rangpur lime	17.3	19.5	11.3	7.7	9.3	17.2
Rough lemon	34	34	0	9.3	10.4	9.6
Volkamer lemon	34.2	35.7	4.2	9.8	11.5	14.8
Sour orange	31.2	33.3	6.3	10.5	10.7	1.9

% Red.= % Reduction (fresh weight of inoculated plant - fresh weight of check)*100
Inoc. = Inoculated with citrus nematode, Check = The rootstock free of nematode

All rootstocks inoculated with nematodes showed reductions in shoot height and root length (Fig. 3.26) when compared to the rootstocks free of nematodes (control). The rates of reductions in shoot height were varied from 0.6 % with Japanese bitter orange to 10.2 % with Rangpur lime. Reduction percentages in root length varied from 3.7 % with Rough lime to 17.2 % with Rangpur lime.

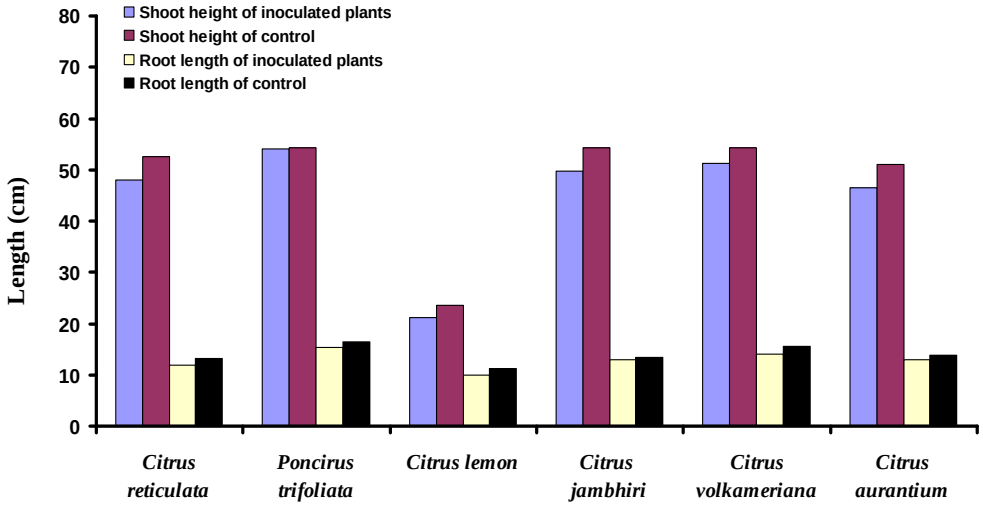


Fig. 3.26: Effect of citrus nematodes inoculation on shoot height and root length of citrus rootstocks

On the basis of shoot and root dry weights (Fig. 3.27), the inoculated rootstocks exhibited reductions compared to rootstocks free of nematodes (control). The rates of these reductions in shoot dry weight ranged from 2.1 % with sour orange to 19.1 % with Rough lemon. Reductions in root dry weight ranged from 2.0 % with Japanese bitter orange to 20.4 % with Volkamer lemon.

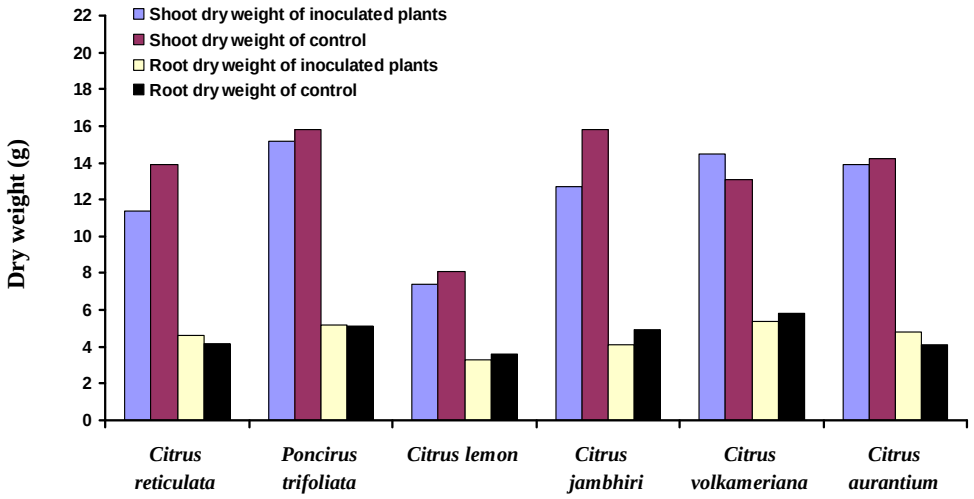


Fig. 3.27: Effect of citrus nematodes inoculation on shoots and roots dry weight of citrus rootstocks

3.3.2 Application of Navel orange scions on resistant rootstocks against citrus nematode

The objective of this study is to evaluate and compare the performance of navel orange scions on resistant rootstocks against the target nematode as new strategy of nematode control.

Navel orange grafted on *Citrus volkameriana* (NOCV) and on *Poncirus trifoliata* (NOPT) were planted and compared to those grafted on sour orange (Control) in their ability to control the plant parasitic nematode *Tylenchulus semipenetrans* under greenhouse conditions. These selections were chosen for their previously achieved high resistance to citrus nematode *T. semipenetrans*.

Data concerning *Tylenchulus semipenetrans* development are presented in Tab. 3.21 and Fig. 3.28 and 3.29. As expected, the citrus nematode exhibited very low reproductive rates on NOCV and NOPT as compared to the control. This trial yielded the best results in controlling citrus nematodes even when using the nematicide at its highest application rate. Furthermore, this trial resulted in significant reductions in all nematode criteria and exhibited a strong decrease in nematode build-up and egg production in all treatments of this study.

NOPT achieved the greatest nematode reductions with counts of 11, 64 and 2301 egg masses, immature stages and final population, respectively. In terms of nematode fecundity, it was observed that NOPT also achieved the greatest reduction in the count of eggs per egg mass, percentage of egg production and eggs per g root with 30, 0.8 % and 30, respectively.

The control recorded the highest rates in the nematode population and fecundity with 235, 539 and 20341 egg masses, immature stages and final population (J_2), respectively and 175, 100 % and 2363 eggs per egg masses, egg production and eggs per g root, respectively.

Tab. 3.21: Reproductivity of *Tylenchulus semipenetrans* influenced by using navel orange scion on resistant rootstocks

Treat.		Nematode counts			Pf Pi^{-1}	Nematode fecundity	
		Egg masses	Immature stages	Final pop. in soil (J_2)		Eggs $eggmass^{-1}$	% egg prod.
NOPT		11^{c}	64^{c}	2301^{c}	0.48^{c}	30^{b}	0.8^{c}
NOCV		36^{c}	134^{bc}	3267^{c}	0.69^{c}	65^{b}	5.7^{c}
Sour orange + Carbofuran ($g\ pot^{-1}$)	**0.5**	112^{b}	193^{b}	6521^{b}	1.37^{b}	96^{b}	26.1^{b}
	1.0	40^{c}	107^{bc}	4053^{c}	0.84^{c}	60^{b}	5.8^{c}
	2.0	36^{c}	104^{bc}	3300^{c}	0.69^{c}	59^{b}	5.2^{c}
Control		235^{a}	539^{a}	20341^{a}	4.22^{a}	175^{a}	100^{a}

Treat. = Treatment, J_2 = second stage juveniles, % Egg prod. = % Egg production, Pf Pi^{-1} = Final population / Initial population,
NOPT = Navel orange on *Poncirus trifoliata*, NOCV = Navel orange on *Citrus volkameriana,*
Control = Navel orange on *C. aurantium* infected by nematodes
Mean values followed by the same letters in column are not significantly different by Tukey's test at 0.05 levels.

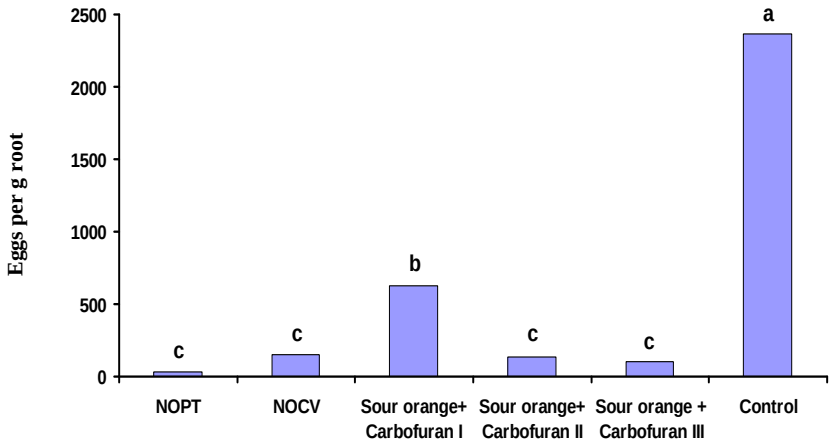


Fig. 3.28: Numbers of eggs per g root recovered from *Tylenchulus semipenetrans* as influenced by navel orange scions on resistant rootstocks

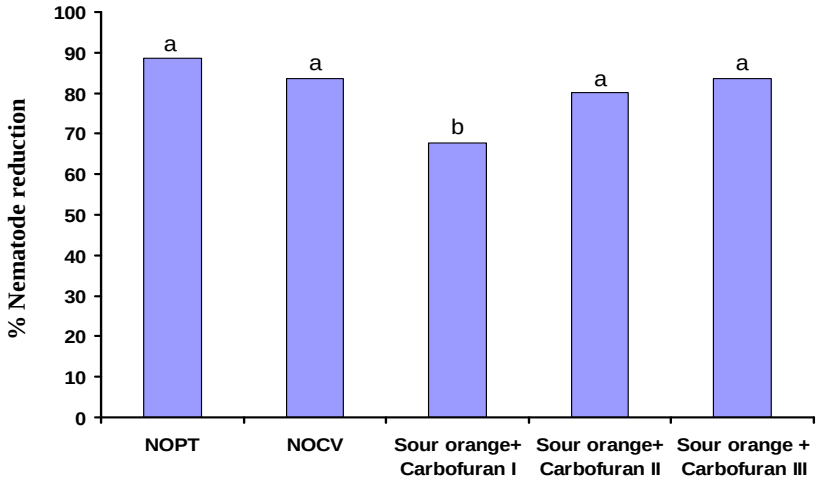


Fig. 3.29: Population reductions of *Tylenchulus semipenetrans* as influenced by navel orange scions on resistant rootstocks

The data in Tab. 3.22 show high increases in plant measurements. These increases were achieved due to the reduction of nematode population as a result of using resistant rootstocks.

With respect to the shoot growth parameters, both NOPT and NOCV achieved increases when compared to control trials. These increases were more noticeable with NOPT, which achieved the highest increases with 52.6 cm in plant height, 42 and 22 g in shoot fresh and dry weight, respectively. NOCV also achieved high increases in all shoot parameters with 51 cm height, 40.4 and 21.9 g in fresh and dry weight, respectively.

NOPT and NOCV also grew in terms of root growth parameters, showing nearly the same trend as was previously observed in shoot growth parameters. NOPT recorded high increases with 26.5 cm length, 21.3 and 9.9 g in fresh and dry weight, respectively. Furthermore, both NOPT and NOCV exceeded effectiveness of the nematicide Carbofuran at doses 0.5 and 1.0 g pot^{-1} in all root growth parameters.

Tab. 3.22: Growth response of navel orange scions on resistant rootstocks infected with *Tylenchulus semipenetrans*.

Treat.	Shoot			Root		
	Height (cm)	Fresh wt. (g)	Dry wt. (g)	Length (cm)	Fresh wt. (g)	Dry wt. (g)
NOPT	52.6[a]	42[a]	22[ab]	26.5[a]	21.3[a]	9.9[a]
NOCV	51[a]	40.4[a]	21.9[ab]	25.7[ab]	20.6[ab]	9.6[a]
Sour orange + Carbofuran (g pot^{-1}) 0.5	49.7[ab]	38.3[a]	24.1[ab]	21[b]	17.2[b]	8.5[a]
1.0	50.5[ab]	39.3[a]	25.2[ab]	23.1[ab]	17.8[ab]	8.9[a]
2.0	53.5[a]	42.4[a]	26[a]	26.4[a]	20.8[ab]	10.5[a]
Healthy plants	51.6[a]	40.6[a]	21.6[ab]	26.4[a]	21.4[a]	10.4[a]
Control	44.9[b]	29.3[b]	20.9[b]	21.9[ab]	17.4[b]	8.3[a]

Treat. = Treatment, NOPT = Navel orange on *Poncirus trifoliata*, NOCV = Navel orange on *Citrus volkameriana*, Healthy plants = Navel orange on *C. aurantium* free of nematode or any treatment, Control = Navel orange on *C. aurantium* infected by nematodes
Mean values followed by the same letters in column are not significantly different by Tukey's test at 0.05 levels.

3.4 The suitable application time of nematode control strategies

Populations of citrus nematodes fluctuate greatly during the year and from one year to the next. These population dynamics are influenced by prevailing environmental conditions. Seasonal cycles of high citrus nematode populations were studied for two years from June 2002 to June 2004. Soil samples were collected at monthly intervals at two different depths of 20 and 50 cm after removing the first 5 cm of soil, from ten citrus trees at the experimental orchards of the Faculty of Agriculture (Moshtohor), Zagazig University, Egypt. These samples were used to determine the population peaks of citrus nematode.

The data presented in Tab. 3.23 showed the seasonal fluctuation of citrus nematodes during one year from June 2002 to June 2003. Data reveal that the nematode population was at its lowest point during the period from June to September, while in November the population tended to show a pronounced build-up (2476 individuals 250 g^{-1} soil), and then decreased slightly in December. A relatively sudden decrease appeared in January and February, followed by a growth period in March and April and a peak in May with 2749 individuals 250 g^{-1} soil.

Tab. 3.23: Seasonal fluctuation of citrus nematode associated with citrus trees in Qaliubiya governorate (2002-2003)

Month		June	July	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June
Nematode count in 250 g soil	Depth (cm) 20	2078	1692	1470	1394	1573	2476	2175	1879	1548	1770	2570	2749	2019
	Depth (cm) 50	1962	1626	1350	1311	1472	2055	1811	1679	1435	1640	2276	2374	1724
Temp. (°C)		34.2	36.9	34.7	33.6	31.0	25.7	21.3	20.2	18.1	20.8	26.6	29.0	35.3

The data presented in Tab. 3.24 show the seasonal fluctuation of citrus nematodes during one year from June 2003 to June 2004. The results show nearly the same trend of fluctuation in population dynamics. Data reveal that a relatively sudden decrease of nematode population appeared in June, as the population decreased rapidly until reaching the minimum in September. This was followed by a sudden increase in October and a maximum in November (3069 individuals 250 g^{-1} soil). The population decreased during January and February, then grew again in March and reached a maximum in April and May with 2290 and 2187 individuals 250 g^{-1} soil, respectively.

Tab. 3.24: Seasonal fluctuation of citrus nematode associated with citrus trees in Qaliubiya governorate (2003-2004)

Month		June	July	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June
Nematode count in 250 g soil / Depth (cm)	20	2019	1742	1367	1352	2651	3069	2227	1629	1786	2070	2290	2187	1781
	50	1724	1550	1052	1005	2228	2454	1705	1467	1549	1759	2002	1892	1669
Temp. (ºC)		35.3	34.7	35.7	32.6	29.4	24.5	22.5	18.1	18.3	22.7	28.1	31.8	34.3

Two seasonal population peaks of the citrus nematode, *Tylenchulus semipenetrans*, were identified from the data gathered (Fig. 3.30). The first population peak occurred in April-May following the spring root flush when soil temperatures become favourable for development; the second peak occurred in October-November, following a fall root flush. It was also noticed that the nematode population at a depth of 20 cm was greater than that of 50 cm among all tested samples and throughout the duration of the experiment. It was noticeable that high temperature degrees reduce nematode population levels.

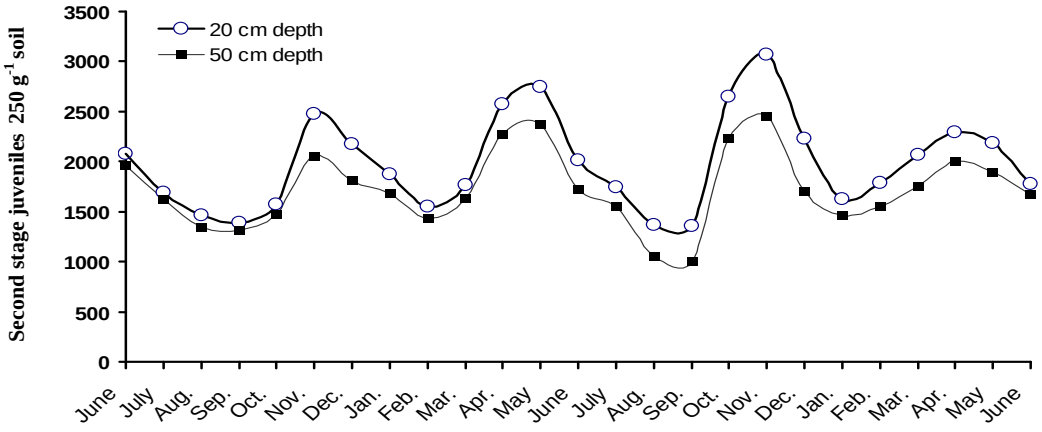


Fig. 3.30: Population density fluctuation of citrus nematode in Qaliubiya governorate (June 2002-June 2004)

4 Discussion

Soil is a vital natural resource essential to civilization, acting as the fulcrum between survival and extinction for most of land-based life. To date, chemical and physical soil properties have been focused on as indicators of soil health more than biological properties have. However, biological processes are strongly correlated to maintenance of soil structure and fertility, and are more sensitive to change than the indicators depending on chemical and physical soil properties.

Maintaining and improving the productive capacity of the soil is essential to human survival and healthy soils are an essential element of this process. Because the physical and chemical components of soils are largely fixed by geographic constraints, the flexibility in soil ecosystems is primarily due to their biological composition and the way this is modified by human inputs (fertilisers, cultivation, plant species, etc.). Furthermore, biological systems are highly sensitive to incipient degradation; hence a change in biological status of the system may provide an 'early warning' of environmental collapse and so allow us to react before irreversible damage occurs (Pankhurst et al., 1997).

Direct measurement of soil health is not possible. Therefore, it is necessary to take measurements of the components or processes of the system that indicate soil health, or more accurately, those that might indicate soil disease (Elliot, 1997).

Nematodes are useful indicators of soil quality and health because of their great diversity and role in many functions of soil food web. Several researchers have introduced methods to evaluate the status of soil quality through the assessment of nematode population. Changes in nematode population reflect changes in soil microenvironment. The use of nematodes as a biological indicator of soil health and quality brings the benefits of using both biotic and abiotic factors and provides an indication of both function and structure of the soil ecosystem. For that purpose, the cyst and citrus nematodes were applied as soil indicators in the present research.

The improvement of sustainable agriculture management systems is complicated by the need to consider their utility to humans, efficacy, and maintenance of the natural balance of the soil community. It is necessary to improve these management systems by balancing the needs for high food production with those for a safe and clean environment.

The use of the integrated pest management ``IPM`` approach for nematodes enables nematode damage to be reduced to tolerable levels through a variety of techniques

including predators, parasites, genetically resistant hosts, environmental modification, culture and nutritional practices and when necessary and appropriate, chemical nematicides. In such system, biological control is a vital and integral part of the IPM procedure and never has to stand alone as a control procedure (Greathead and Waage, 1983).

The primary emphasis of this work was enhancing soil antiphytopathogenic potential against the most economically important plant attacking nematodes, sugar beet cyst nematode *Heterodera schachtii* in Germany and citrus nematode *Tylenchulus semipenetrans* in Egypt, using safe methods with an emphasis on improvement of soil health and quality. To achieve this goal some assessments and experiments were designed. Firstly, some assessments were conducted under field conditions to evaluate the population density and dynamics of plant parasitic nematodes of navel orange trees in Qaliubiya Governorate, Egypt. Secondly, greenhouse experiments were designed in Egypt and Germany to study the following:

- Improvement of soil health and quality against plant parasitic nematodes depending on the cyst and citrus nematodes as indicators using:
    - *i.* Organic and inorganic fertilisers
    - *ii.* Biological control
    - *iii.* Plant extracts
- Evaluation of different citrus rootstocks as a mean to control citrus nematodes.

4.1 Evaluation of the approaches used to enhance the antiphytopathogenic potential of soil against the sugar beet cyst nematode *Heterodera schachtii* and the citrus nematode *Tylenchulus semipenetrans*

Organic amendments

The basis of sustainable nematode control is the maintenance of a healthy soil food web. This begins with routine application of organic matter. There is substantial evidence that the addition of organic matter in the form of compost or manure will decrease nematode pest populations and associated damage to crops (Stirling, 1991b). This could be a result of improved soil structure and fertility, alteration of the level of plant resistance, release of nematotoxins, or increased populations of fungal and bacterial parasites and other nematode-antagonistic agents (Akhtar and Malik, 2000). Higher organic-matter content increases soil water-holding capacity, and supports thriving communities of the decomposers and predators that make up the soil "digestive system".

The nematode control achieved by decomposition of plant residues in soil was found to be due to liberation of fatty acids (Montasser, 1991; El-Naggar et al., 1993). The high contents of fatty acids of botanical meals or oils are the expectant chemicals produced during decomposition of such chemicals and affect nematodes ecology. Accumulated toxins from decomposed products (Johnson, 1974; Alam et al., 1973) and tannins into nematotoxic polyphenols (Tayler and Murant, 1966) increase host resistant to nematode infection. Also, rapid decomposition of organic matter causes nematode death (Hollis and Rodriguez-Kabana, 1966) due to a rapid increase of butyric and propionic acids (Kesba, 2003). Furthermore, the microbial breakdown of nitrogen containing substances in soil via processes of mineralisation and nitrification might be effective against nematodes by increasing predacious nematodes, nematode-trapping fungi or their toxins. NH_3 or possibly nitrite production are among principle compounds responsible for the decreased nematode populations (Walker, 1992). Compost is effective because it fosters a more diverse soil environment in which a myriad of soil organisms exist. Compost acts as a food source and shelter for the antagonists that compete with plant pathogens, for those organisms that prey on and parasitise pathogens, and for those beneficials that produce antibiotics (Harrison and Frank, 1999). The major difference between compost-amended soil and the other techniques is that organic matter in compost is already "digested." Other techniques require the digestion to take place in the soil, which allows for both anaerobic and aerobic decomposition of organic matter. Properly composted organic matter is digested chiefly

through aerobic processes. These differences have important implications for soil and nutrient management, as well as plant health and pest management (Sullivan, 2004). Goldstein (1998) confirmed that systemic resistance was also induced in plants in response to compost treatments: composts and compost teas do activate disease resistance genes in plants. These disease- resistant genes are typically “turned on” by the plant in response to the presence of a pathogen. These genes mobilise chemical defences against the pathogenic invasion, although often too late to avoid the disease. In plants growing in compost, however, these disease-prevention systems are already running. Sullivan (2004) described the Induced resistance as pathogen-specific, but it does allow an additional way to manage certain diseases through common farming practices

In this paper, the addition of waste materials from plants to soil has been explored as an alternative means of nematode control. These materials include agricultural wastes in the form of commercial potting mixture (Tab. 3.1 and Fig. 3.1 - 3.3) or crushed garlic (Tab. 3.9, Fig. 3.13 and 3.14) were examined in controlling the concerned nematodes. The results emphasised that the tested organic amendments were pestilential to populations of cyst and citrus nematodes with respect to their nature, origin, concentration and method of application. They yielded significant reductions in nematode criteria compared to control values with positive response in plant growth (Tab. 3.2 and 3.10). Potency of these materials on nematode population was comparable to the comparable nematicide Carbofuran.

The beneficial effects of organic amendments are generally considered to cause by direct or indirect stimulation of predators and parasites of plant-parasitic nematodes: when there was a decrement in the soil-pathogen population, there was a consequent increment in crop yield. Based on this context, it is suggested that the incorporation of organic amendments and antagonistic fungi can inhibit nematodes more effectively than individual application of either treatment.

The mode of action of organic amendments leading to plant disease control and stimulation of microorganisms is complex and dependent on the nature of the amendments (Akhtar and Abdul Malik, 2000). Organic fertilisers provide organic matter to the soil for growth and development of predatory nematodes (Akhtar, 1998). Application of soil amendments and use of novel nematicidal compounds derived from microbial culture filtrates were used as new management tactic for controlling plant parasitic nematodes. Field and green house studies demonstrated that this tactic reduced nematode numbers, and limited subsequent crop losses (Kaplan and Noe, 1992; Riegel et al., 1996 and Noe, 1998).

The suppression of nematodes obtained by treatment with animal manures increased significantly by inoculation of litter or manures with specific fungi that act as biocontrol agents for plant parasitic nematodes (Riegel and Noe, 2000). Soil fauna can enhance soil organic matter decomposition and nutrient mineralisation. Free-living (microvorous) nematodes both contribute to decomposition processes of organic soil amendments and increase the mineralisation of nitrogen and phosphorus (nutrient cycling), and may have an indirect beneficial effect on plant growth (Yeates and Colemann, 1982; Ingham et al., 1985).

Many researchers have applied biological wastes to control phytopathogenic nematodes and supported the results obtained from the present study. For instance, Chicken litters, pigeon excrements, poultry droppings and rabbit dung achieved significantly reduction of the nematodes infecting cowpea (Osman et al., 1989). Similar results were achieved on potato tuber yield and *Globodera pallida* population density (Sarker and Main, 1995 and Maareg et al., 2000). Chicken manures were tested against the cereal cyst nematode, *Heterodera avenae* under field conditions and suppressed nematode reproduction by reducing the number of cysts on wheat roots and in soil (Al-Mihanna et al., 1999). Chicken and pigeon litters significantly reduce root galling and number of egg masses on tomato roots (Al-Rehiayani, 2001). Addition of pigeon excrements or peanut meal to the soil exceeded the effect of the nematicide Vydate 10 % in reducing the nematode final population (Kesba, 2003).

In this paper, the addition of waste materials from animals to soil has been applied as a mean of nematode management. These biological wastes in the form of pigeon excrements (Tab. 3.9, Fig. 3.13 and 3.14) were against the growth of citrus nematode population with respect to their concentration and method of application. They showed significant reductions in nematode counts and fecundity compared to the control. Positive response in plant growth was achieved (Tab. 3.10). The effect of the pigeon excrements on citrus nematode populations was comparable to the comparable nematicide Carbofuran and seemed to be dependent on its concentration.

Inorganic fertilisers

Ammonial fertilisers (urea, ammonium sulphate, ammonium nitrate, ammonium chloride), which are subjected to nitrification, were also effective in killing plant pathogens in soil (Lazarovits et al., 2000). It has long been known that the form of nitrogen fertiliser can influence plant disease incidence. When the roots absorb nitrate nitrogen, an alkaline

root zone condition is created. On the other hand, when the roots absorb ammonium nitrogen, an acid root zone is created (Sullivan, 2004). A more acid soil also fosters better uptake of manganese. Adequate manganese stimulated disease resistance in some plants. The uptake of ammonium nitrogen improved plant uptake of manganese and decreased diseases (Woltz and Jones, 1973). Cyst nematodes do not hatch well in acid soils or alkaline soils. They do best in soil with a near-neutral pH (Yepsen, 1984). This can be used to some advantage. For example, sugar beets may be safest from nematode damage in an acid soil, which contains higher amounts of ammonium nitrogen or commercial potting mixture.

The modes of action proposed for high N-amendments include generation of toxic volatiles (Huang and Sun, 1991), soil anoxia (Block et al., 2000), and promotion of biological control agents (Dutta and Isaac 1979, Lumsden et al., 1983). The mechanism most often implicated, however, is ammonia (NH_3) production. Ammonia was found to be toxic to pathogens in solution assays (Oka et al., 1993, Punja and Grogan 1982) and as a volatile emanating from amended soils (Chang and Chang, 1999, Huang and Janzen, 1991).

In the present research, use of OMF* and ammonium nitrate as inorganic fertilisers achieved high results in both nematode suppression and plant growth response. For OMF, the highest effectiveness in reducing the number of sugar beet nematode cysts, total population and population build-up was achieved at the recommended dose (100 %). Its effectiveness decreased at higher or lower doses (Fig. 3.1- 3.3). At the same concentration, significant increases in plant growth response were detected compared to control (Tab. 3.2). The OMF recorded increases in plant growth more than double compared to the control values and exceeded significantly the effect of the nematicide Carbofuran. Referring to ammonium nitrate, the inhibition of nematode counts and fecundity increased steadily by increasing the doses of NH_4NO_3 (Tab. 3.9, Fig. 3.13 and 3.14). The same trend was achieved in plant measurements (Tab. 3.10). Ammonium nitrate recorded significant increase in plant shoot and root measurements compared to all comparable treatments (Control, healthy plants and Carbofuran)

* Optimum mineral fertilisation

Amino acids and organic acids

Efficacy of amino acids in reducing nematode population differs according to their isomers and host plant as well as nematode species. With regard to the mode of action, the most reliable evidence was indirect: amino acid metabolites are concentrated at root tips, which are initial feeding sites of the nematodes, thereby blocking essential metabolic pathways in either the plant or nematode and interfering with nematode nutrition (Parasad and Webster, 1967; Evans and Trudgill, 1971). This delaying the total life cycle, the triggering mechanism of sex hormone production or reduces the number of giant cells incited by nematodes, which in turn affect the nutrition of the nematodes (Parvatha-Reddy et al., 1975). Amino acids may activate praline hydroxylation that reverses the susceptible plants to resistant (Giebel and Krenz, 1975). The mechanism could be explained as ability of hydroxyproline to induce cessation of cell wall extensibility and retardation of auxin action (Norris, 1967). Accumulation of proteins in the roots may reach toxic limits due to amino acid spray application and may have a direct toxic effect (Kesba, 2003). It has been suggested that D-forms of the amino acids could act as antimetabolites in nematodes (Prasad and Webster, 1967) and compete for enzymes with the normal L-amino acid substrates. However, D, L and DL forms were equally effective in preventing reproduction of nematodes. For instance, effects of methionine appear to be independent of the isomeric form. The addition of methionine ingested caused inhibition effect on oxygen metabolism within the nematode. (Evans and Trudgill, 1971). Ammonia and urea were shown to suppress several nematode species in tests, and the decomposition of some forms of organic nitrogen, (and organic sulphur in the case of methionine) can release products that are toxic to nematodes (Rodriguez-Kabana, 1986; Oka and Pivonia, 2002). The direct influence of methionine against the root-knot nematode *Meloidogyne incognita* was tested. Egg hatching and the number of active juveniles were reduced in 0.25 g litre^{-1} DL-methionine solutions after 48 h of treatment when compared with juveniles kept in water. Reduction in egg hatching was 48.4 % after 4 days in 0.25 g litre^{-1} methionine solutions (Talavera and Mizukubo, 2005). Further research, however, is necessary to determine the precise mode of action of this amino acid.

Many researchers supported the findings of the present research. For instance, foliar spray of L-arginine and L-glutamic acids suppressed the numbers of root galls, females and egg masses on either susceptible or resistant tomatoes (Al-Sayed and Thomason, 1988; Al-Sayed, 1992). The amino acids as a foliar spray were not observed to have an adverse effect on the growth and vigour of tested plants, but significantly affected

the development and reproduction of root-knot nematode *M. incognita* (Krishna-Prasad and Setty, 1974). Amino acids were significantly effective in reducing the root-knot nematode *Meloidogyne incognita*, numbers of galls, egg masses, egg production and nematode build-up. The degree of effectiveness depends on its concentration (Kespa, 2003).

Organic acids have contact nematicidal action on free stages of parasitic nematodes (Al-Sayed et al., 1988). These acids may play direct or indirect role in biological defence mechanisms since they increase proteins and fatty acids in root tissues (Kespa, 2003). Salicylic acid (SA) is known to play a critical signalling role in the activation of plant defence responses after pathogen attacks (Klessig et al., 2000). SA injected into stems, sprayed onto the entire plant, or applied to the roots acts as a potent inducer of systemic resistance and pathogen-related (PR) proteins (Ward et al., 1991; Enyedi et al., 1992; Leeman et al., 1996). Therefore, SA production by bacteria in the rhizosphere may enhance defence mechanisms in plants leading to systemic resistance against a variety of soil-borne plant pathogens including plant-parasitic nematodes (Siddiqui and Shaukat, 2004).

In the present work, the amino acids achieved significant reductions in nematode criteria followed by the organic acids, ascorbic and salicylic (Tab. 3.11, Fig. 3.15 and 3.16). Both organic acids showed steadily reductions in nematode counts and reproduction when increasing their doses. Positive relations were observed between the applied acids and plant growth measurements (Tab. 3.12). All levels of Amino and organic acids achieved increments in plant measurements.

Microorganisms

Fungi

Among the organisms that are most likely favoured by cover crops are fungal egg-parasites, nematode-trapping fungi, endoparasitic fungi, endomycorrhizal fungi, plant-health promoting rhizobacteria, and obligate bacterial parasites (Sikora, 1992). Nematophagous fungi include a wide and diverse range of fungi which parasitise nematodes. They can be divided into four categories: endoparasitic fungi, nematode-trapping fungi, fungi which parasitise eggs and females, and toxin-producing fungi (Barron and Thorn, 1987; Dackman et al., 1992). In some aspects, fungi are preferable to vascular plants as source of naturally occurring antinematodal compounds because of the less complex anatomical structure of fungi and their adaptability to being grown in large

fomenters. A logical place to initiate the isolation of nematode-antagonistic fungal compounds would be with nematode-antagonistic fungi (Twomey et al., 2000).

Several *Trichoderma* strains have been reported to be effective in controlling plant diseases. The action of fungal hydrolytic enzymes is considered to be the main mechanism involved in the antagonistic process. A protease produced by *Trichoderma harzianum* was purified to homogeneity by precipitation with ammonium sulphate followed by hydrophobic chromatography. Western-blot analysis showed that the enzyme was present in the culture supernatant 24 h after the *Trichoderma* started to grow in casein-containing liquid medium. The capacity of *Trichoderma harzianum* protease to hydrolyze the cell wall of the host indicates that this enzyme may be actually involved in the antagonistic process. This strongly suggests that hydrolytic enzyme over-producing transgenic fungi may show superior biocontrol capacity (De Marco and Felix, 2002).

Mycoparasitic *Trichoderma* strains secrete a complex set of hydrolytic enzymes under conditions related to antagonism. Several proteins with proteolytic activity were detected in culture filtrates from *Trichoderma harzianum*. The protein responsible for the main activity (PRA1) was purified to homogeneity. The number of hatched eggs of the root-knot nematode *Meloidogyne incognita* was significantly reduced after incubation with pure PRA1 preparations. This nematicidal effect was improved by using fungal culture filtrates, suggesting that PRA1 has additive or synergistic effects with other proteins produced during the antagonistic activity of *T. harzianum*. (Suarez et al., 2004). Acetic acid was identified as nematicidal compound in culture filtrate of *Trichoderma longibrachiatum*. The activity was as low as 20 μg ml^{-1} against cyst nematode (Djian et al., 1991).

Arthrobotrys oligospora was the most frequently detected species among the nematode-trapping fungi, and it was found in around 70 % of the sprinkled plates both from the rhizosphere of the different crops and in the root-free soil (Persmark and Jansson, 1997). Researchers have proposed that *Arthrobotrys oligospora* and related fungi trap soil nematodes attempting to obtain nitrogen and thereby compete saprophytically for carbon and energy in nitrogen-poor environments, including litter and wood. When the soil nitrate level is high, it is possible that nematode trapping in soil might not be stimulated (Jaffee, 2004).

Studies were conducted to determine the in vitro tolerance of the nematophagous fungus *Arthrobotrys oligospora,* to the fungicides chlorothalonil and myclobutanil used to manage diseases on putting greens; the concentration of fungicides obtained from

simulated putting green soil; and the ability of the fungus to reduce populations of the ring nematode, *Criconemella ornata*. Both fungicides reduced in vitro hyphal growth and germination of conidia above 10 mg kg^{-1}. Soil concentrations of chlorothalonil were less than 5 mg kg^{-1} and concentrations of myclobutanil were below detection limits. Nematode populations were not affected by *A. oligospora* in simulated greens however nematode populations were lowest in inoculated pots (Woodward et al., 2005). *A. oligospora* was the only species of the nematophagous fungi tested that grew chemotropically towards root tips from all three plants tested. The directed growth towards roots evident in *A. oligospora* may explain the higher abundance of this fungus in the rhizosphere than in root-free soil (Persmark and Jansson, 1997). A positive rhizospheric effect on *Arthrobotrys* spp. was also reported for citrus (Gaspard and Mankau, 1986)

Linoleic acid was shown to be the only detectable nematicidal agent in the mycelial extracts of several predacious fungi of the genus *Arthrobotrys* (Anke et al., 1995). Linoleic acid exhibited nematicidal activities towards the free-living nematode *Caenorhabditis elegans* and *Meloidogyne incognita* with LD50 values of 5 and 50 µg ml^{-1}, respectively (Anke and Sterner, 1997).

Certain strains of *Fusarium* fungi, when grown in laboratory cultures, secrete compounds that are toxic to plant-feeding nematodes (Meshram and Goswami, 1989; Hallman and Sikora 1996). As part of a study searching for biological control agents, culture broths of over 250 fungi isolated from soybean cyst nematode (*Heterodera glycines* Ichinohe) were screened for nematode antagonistic activity (Meyer et al., 1998). Fusarium species were common among those isolates producing broth filtrates, which inhibited in vitro egg hatching of soybean cyst nematodes and root-knot nematodes, *Meloidogyne incognita*. One of these strains, an isolate of *Fusarium equiseti*, was especially active against *M. incognita* (Nitao et al., 1999).

Different compounds in culture filtrates of *F. equiseti* (Corda) Sac. may antagonise nematodes (Nitao et al., 2000). The fungus *Fusarium equiseti* isolated from the cyst nematode secretes nematode-antagonistic compounds. Bioassay-guided fractionation of an extract of the culture broth was performed to identify the compounds. Fractions were assayed for activity against a root-knot nematode (*Meloidogyne incognita*). Two trichothecene compounds were isolated that inhibited egg hatch and immobilised second-stage juveniles of this nematode: 4,15-diacetoxy-12,13-epoxy-3,7-dihydroxytrichothec-9-en-8-one (4,15-diacetylnivalenol) and 4,15-diacetoxy-12,13-epoxy-trichothec-9-en-3-ol (diacetoxyscirpenol). This is the first published report of these compounds affecting plant-

parasitic nematodes (Nitao et al., 2001). The cyclic depsihexapeptide enniatin A was found in the culture filtrate of *Fusarium* as nematicidal compound against *C. elegans* and *M. incognita* (Mayer et al., 1999).

Verticillium sp. originally isolated from *M. incognita* eggs was fractionated with in vitro toxicity and root invasion bioassay. The nematicidal compound, Phornalactone (C8), was active at 500 µg ml^{-1} (Khambay et al., 2000).

In the present research, all applied fungi filtrates and bacteria significantly impaired the nematode counts and reproductivity compared to the control. The inhibition of the nematode counts and fecundity increased steadily with increasing the filtrate concentration. Differences in nematode suppression were noticeable among fungi genera, where the highest reduction in cyst nematode was achieved by *Fusarium equiseti* (Tab. 3.3, Fig. 3.4-3.6) while *Nematoctonus concurrence* achieved the highest rate of citrus nematode reduction (Tab. 3.13, Fig 3.17 and 3.18). All fungi filtrates achieved increases of plant measurements or at least were not harmful to the plant (Tab 3.4 and 3.14).

Based on the present findings, it could be confidently stated that the present work reveals promising agricultural soil methods of non-conventional and chemical nematode-management.

Plant extracts

The present study was conducted for testing the nematicidal activities of *Thuja orientalis* and *Thevetia neriifolia* extracts using the two solvents ethyl acetate and hexane in controlling citrus and cyst nematodes under greenhouse conditions. The solvents were evaporated under reduced pressure

There are reports that certain plant parts and extracts possess nematicidal properties (Sharma and Trivedi, 1992). Application of these plant parts or extracts to nematode infested soil affects nematodes directly and stimulates soil microbes that reduce nematode population (Reddy et al., 1996). These plants have yielded a broad spectrum of compounds which are active toward plant-parasitic nematodes, including polythienyls, isothiocyanates, glucosinolates, cyanogenic glycosides, polyacetylenes, alkaloids, lipids, terpenoids, sesquiterpenoids, diterpenoids, quassinoids, steroids, triterpenoids and simple and complex phenolics (Chitwood, 2002).

The nematicidal properties of some of these plants were tested in the laboratory against plant parasitic nematodes. For instance, extracts of neem and datura and their

subsequent dilutions were studied on the larvae mortality of citrus nematode in laboratory, yielding significant results (Ahmed et al., 2004).

Thuja orientalis

Many reviews have been published about the mode of action and clinical uses of *Thuja orientalis* as well as its applications in controlling insects. The fresh plant (in relation to the dry substance) contains 0.6 % essential oil, 2.07 % reducing sugar, 4.9 % water-soluble polysaccharides, 2.11 % water-soluble minerals, 1.67 % free acid and 1.31 % tannic agents. The essential oil of the fresh leaves (related to the monoterpene fraction) contains 65 % thujone, 8 % isothujone, 8 % fenchone, 5 % sabines and 2 % α-pinen as the main monoterpenes (Harnischfeger and Stolz, 1983). Other monoterpenes, namely carvotanacetone, origanol, origanes, myrcen and camphen have been described (Kawai et al., 1994). Recently, further bioactive constituents have been found (Chang et al., 2000). High molecular weight glycoproteins/polysaccharides are highly relevant for the activity of the plant (Neth et al., 1995). Analysis of volatile oil carried out by gas chromatography on seasonal samples of volatile oil extracted from the leaves and twigs and the cones of *Thuja orientalis* showed the presence of following substances: thujone (the main constituent: 65 %), fenchone, borneol, limonene, pinene, camphor, myrcene. (Mabey, 1988). The volatile oil that found in the plant has also showed antimicrobial properties (Bagci and Digrak, 1996).

Thuja orientalis is closely related to *Thuja occidentalis* L. In fact, properties attributed to the *T. occidentalis* are also attributed to the *T. orientalis* (Grieve, 1994). Phytochemical studies on *T. occidentalis* have resulted in the isolation of several diterpenes (dehydroabietane, neothujic acids III and IV), (Witte et al., 1983) lignans [(-)-matairesinol, (-)-thujaplicatin methyl ether, (-)-wikstromol, epi-pinoresinol], (Kawai et al., 1999) monoterpenes (α-thujone, β-thujone, fenchone) (Svajdlenka et al., 1999) and a sesquiterpene alcohol [(+)-occidentalol] (Amano and Heathcock, 1972).

Hexane solvent is suitable for dissolving the monoterpenes and it is volatile enough to allow reducing the volume of the hexane fraction without appreciable losses of the essential oil components (Chizzola et al., 2004). An ethyl acetate-soluble extract of the combined leaves and twigs of *Thuja occidentalis* was prepared. Bioassay-guided fractionation of this extract led to the isolation of six active constituents (Fig. 4.1), namely, (+)-7-oxo-13-epi-pimara-14,15-dien-18-oic acid (1), (+)-7-oxo-13-epi-pimara-8,15-dien-

18-oic acid (2), (+)-isopimaric acid (3), (1S,2S,3R)-(+)-isopicrodeoxypodophyllotoxin (4), (-)-deoxypodophyllotoxin (5), and (-)-deoxypodorhizone (6) (Chang et al., 2000).

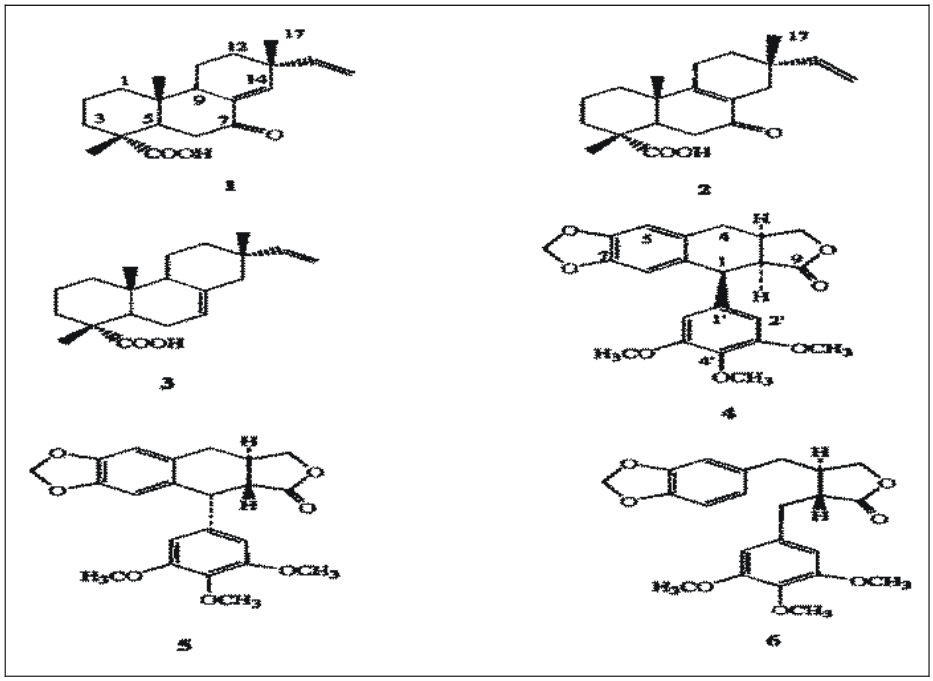


Fig. 4.1: Chemical structure of bioactive compounds derived from ethyl acetate-soluble extract of the combined leaves and twigs of *Thuja occidentalis*

Most plant nematological studies have begun with plants or plant compounds known to be active against other pests and pathogens. Nonetheless, the few investigations of phytochemicals, which are biologically active against nematodes, have yielded a wide variety of structurally diverse compounds (Chitwood, 2002). The essential oil of *Thuja orientalis* was tested in its vapour form against the coleopteran insect *Acanthoscelides obtectus*. The tests revealed that *Thuja orientalis* has a repellent action, reduces fecundity, decreases egg hatchability, increases neonate larval mortality and adversely influences offspring emergence (Papachristos and Stamopoulos, 2002)

GC/MS analysis of *Thuja orientalis* essential oils recorded the presence of 65 peaks. The most abundant compounds have been identified. These identified oils were alpha-cedrol, Lenalool, beta-carrophllene, germacrene-d, germacrene-b, camphene, alpha-phellandrene, limonene, beta-phellandrene, alpha-elemene, (+)-2-carene, valencene, beta-pinene, sabinene and T-murolol. α-cedrol was the most abundant oil with 14.46 % followed by β-carophyllene (7.91 %), germacrene-D (6.05 %), germacrene-B (4.5 %), camphene (3.97 %), α-phyllanrene (3.37 %) and other constituents of the oil sample (Roshdy, 2005).

Thevetia neriifolia

Thevetia neriifolia is highly toxic and induces a series of behavioural autonomic and metabolic alterations that can be lethal. Further studies are needed in order to characterise these effects as well as to isolate from the plant the components that are responsible for toxic activity (Enriquez et al., 2002). A few research projects were conducted to determine and isolate the compounds responsible for this toxicity. In the present study the extracts of *Thevetia neriifolia* demonstrated that high toxicity against *Tylenchulus semipenetrans* and *Heterodera schachtii*, this toxicity may indicate the existence of some nematotoxic compounds in the crude extract.

C-nor-D-homo-homologues of cannogenin and uzarigenin glycosides were isolated along with known cardenolide glycosides from the frozen fresh leaves of *Thevetia neriifolia*. A bisdesmosidic tetraoside of 3 beta,14,21-trihydroxy-5 beta,14 beta-pregnan-20-one was also obtained from the polar fraction and the structure established (Abe et al., 1994).

The results of Bai and Koshy (1999) support the findings of the present research. They stated that leaf extracts, or seed extracts, of *Thevetia neriifolia* using acetone, benzene, ethanol, hexane, methanol or water as extractants reduced the infection of the bittergourd [*Momordica charantia*] leaves by the insect *Henosepilachna vigintioctopunctata.* They added that seed extracts provided the highest leaf protection. The highest leaf protection was observed when using an ethanol extraction. Seed and leaf extracts obtained by crude extraction using ethanol exhibited high antifeedant activity.

In this context, it was found that the use of plant extracts with nematicidal properties could be an effective, cheap and safe nematode control measure. In the present study, it was clearly observed among *Heterodera schachtii* (Tab. 3.5-3.8 and Fig. 3.7-3.12) and *Tylenchulus semipenetrans* (Tab. 3.15-3.18 and Fig. 3.19-3.22) that:

- Crude extracts of both *Thuja orientalis* and *Thevetia neriifolia* achieved high nematicide activity and showed great reductions in nematode populations without harmful effects to the plant health especially when extracted by the solvent ethyl acetate.
- Results obtained from application of botanical extracts were equally or less effective than the comparable nematicide Carbofuran in reducing nematode reproductivity.

- The extracts of *Thuja orientales* yielded higher rates in both nematode reductions and plant measurements compared to *Thevetia neriifolia* extracts.
- The best results in nematode reductions were achieved when both crude extracts were used at concentration of 5 %.
- It is assumed that the effect of solvents is negligible because the treated nematodes and plants are never in direct contact with these solvents.

No research work in the available nematode literatures has been found so far, to support or disprove the obtained findings as a result of the two plant extract applications.

The implementation of plant extracts in integrated pest management (IPM) can be achieved using a soil treatment, which combines biocides and fertilisers. Biological control activity may be increased by combining two (or more) types of bioagents.

4.2 Evaluation of different rootstocks as a mean to control citrus nematodes

Nematologists are now focused on alternative control strategies, including cultural methods. Resistant rootstocks are now considered to be the most direct and effective solution among nematode management options.

Sour orange (*Citrus aurantium*) was formerly the most widespread citrus rootstock. In Spanish orchards more than 95% of the trees were grafted on this rootstock (Cambra, 1994). At present more than 80 % of the citrus trees produced in Spanish nurseries are grafted on the tolerant rootstock Carrizo citrange (*Citrus sinensis* x *Poncirus trifoliata*) (Forner and Pina, 1992). Resistance to *T. semipenetrans* is derived from *P. trifoliata* and it seems to be dominant and oligogenic (Hutchinson, 1985). The selection of Cleopatra mandarin x *P trifoliata* significantly limited reproduction of a Mediterranean bio-type of *T. semipenetrans* after exposure to a range of inoculum densities in field microplots (Galeano et al., 2003). Cleopatra mandarin is considered to be moderately resistant against citrus nematodes (Davis, 1984). The selection of *Citrus volkameriana* x *P. trifoliata* showed very low infectivity and reproductive potential against citrus nematodes and was considered to be resistant (Verdejo et al., 2000a). Rough lemon and Rangpur lime roots generally support a higher population of citrus nematode than sour orange roots and are considered to be susceptible rootstocks (O'Bannon, 1968; Mani, 1989). On the other hand, results published by Hutchinson et al. (1972) reported that *P. trifoliata* lines have also been used as a source of resistance to the citrus nematode in the selection of hybrids between Citrus species and *P. trifoliata*. Some of these hybrids have been recommended to replace commonly used citrus rootstocks, such as rough lemon in Florida.

The results of the present study (Tab. 3.19-3.22 and Fig. 3.23-3.29) follow the same trend of the other similar studies except for the study of Hutchinson et al. (1972) that suggests replacing *P. trifoliata* with rough lemon in case of the hybrids between *Citrus* species and *P. trifoliata*. That disagreement is due to the high effectiveness of *P. trifoliata* to reduce nematode population. According to the obtained data, it was classified as ``high resistant``, while the rootstock rough lemon was classified as ``highly susceptible`` to citrus nematodes. The combined use of nematode-free and resistant rootstocks will prevent or restrict citrus nematode populations, reducing citrus crop losses and grove production costs associated with other types of nematode management.

The mechanism of resistance needs to be determined. This resistance can be biochemical or structural. Biochemically, roots can incorporate polyphenols in the walls of the epidermis that could be involved in disease resistance (Tippet and O'Brien, 1976; Duncan et al., 1993). Structurally, roots can develop thick, cellulosic walls that can be lignified to counter the tendency to collapse under stress (Peterson and Waite, 1996). In the exodermis, lignin and subrinlayers can develop which protect the root interior after the epidermis is sloughed away (Walker et al., 1984). Nematode reproduction on the resistant rootstocks is unfavourable because of decreased female fecundity, higher proportion of males (Verdejo L. et al. 2000b), and higher accumulation of deposits lignin-suberin like material (Galeano et al., 2003). Cell wall thicknesses of cortical and stellar tissues in a radial transect of transverse section of first-order fibrous roots of sour orange and trifoliata orange were measured. The cell wall thicknesses of trifoliata orange were thicker (0.17 and 0.46 μm) than those of sour orange (0.15 and 0.41 μm) in cortical and stellar tissues, respectively (Eissenstat and Achor, 1999). Differences in expression of resistance suggest that there is one major gene or multiple tandem genes directly guiding to *T. semipenetrans* resistance (Ling et al., 2000).

4.3 Determination of the most suitable application time for nematode control strategies

Understanding the interaction between citrus roots and pest population growth is critical to the development and implementation of sound, biologically based, pest control practices. Citrus root growth is greatly affected by environmental factors. Three distinct cycles of root growth have been observed in the growth of mature trees. The first root flush usually occurs between late February and early April, the second occurs from May to June and the third from August to October. During spring, summer, and fall, alternation of root

and shoot growth has been repeatedly observed. This alternation of growth is thought to occur as a result of competition between roots and shoots for photosynthetic carbohydrates.

Similar to fibrous root growth, two seasonal population peaks of the citrus nematode *Tylenchulus semipenetrans* are known to occur in mature citrus. Nematode population cycling is closely associated with and commonly follows major spring and fall periods of root flushing. Root flushing and pest population cycling are closely interrelated because susceptible root tissues are produced prior to or during periods of soil temperatures leading to nematode population development. The first population peak usually occurs in April to May, following the spring root flush when soil temperatures become favourable for development, and again from October to November, following a fall root flush. Populations of citrus nematodes, like fibrous root growth, are usually at low levels during winter, from late December through February (Noling, 2002). The population varies in relation to depth and month of sampling. Fall populations (November-December) are generally higher than spring populations (April-May) due to the higher numbers of nematodes found in late summer than in late winter resulting in a greater reproductive potential for the fall population (O'Bannon and Stokes, 1978).

Results of the present study (Tab. 3.23, 3.24 and Fig. 30) are in agreement with Noling (2002) except for the time of fall population, which appears to be during October-November instead of November-December. That could be due to the more favourable temperature of citrus nematode reproduction achieved in October and November, or perhaps the high temperatures in summer allow the fall root flush to be earlier.

All strategies included in this research were practicable and economically suitable. Furthermore, they supported soil health and quality, which reflected an enhancement of plant measurements as well as creating an unsuitable soil environment for nematode feeding and reproduction.

5 Summary

The key objective of the present research work was to enhance the soil antiphytopathogenic properties against the most economically important plant attacking nematodes, the sugar beet cyst nematode *Heterodera schachtii* in Germany and the citrus nematode *Tylenchulus semipenetrans* in Egypt. The applied methods were performed with an emphasis on improvement the soil health and quality. That was realised in field and greenhouse experiments during 2002-2005.

Three main questions were answered:

1. How might soil quality and health be maintained or improved through agricultural and biological management?

By organic and inorganic amendments:

- Commercial potting mixture at all application levels yielded significant reductions in all cyst nematode measurements and exhibited acute decreases in eggs per cyst.
- Pigeon excrements at all levels yielded high reductions in all citrus nematode measurements and exhibited acute decreases in nematode build-up and egg production. Resembling results were achieved using crushed garlic.
- The liquid fertiliser OMF* was highly effective in numbers of cysts and affected cyst nematode counts and reproductivity.
- The application of NH_4NO_3 decreased the population and fecundity of citrus nematode. This nematode population reduction was stronger with increasing of the fertiliser dose.
- Amino acids achieved high reductions in citrus nematode final population. Proportional relations were observed between increasing the doses of organic acids (ascorbic acid and salicylic acid) and recessive citrus nematode reproduction.

By biological control:

- The filtrates of *Arthrobotrys oligospora*, *Fusarium equiseti*, *Trichoderma harzianum* and *Verticillium lecanii* possessed nematicidal activities against cyst nematodes. All fungi filtrates without dilution performed better than the effect of the comparable nematicide Carbofuran even at its maximum rate and achieved significant reductions in all nematode counts.

* Optimum mineral fertilisation

- Filtrates of *Fusarium equiseti* yielded the maximal effect in controlling cyst nematode, followed by *Verticillium lecanii, Arthrobotrys oligospora* and finally *Trichoderma harzianum*.
- Filtrates of *Nematoctonus concurrence* and *Dactylella brochopaga* yielded strong reduction in all citrus nematode counts. It was observed that the inhibition of nematode counts and fecundity was increased, especially when the filtrates applied at their maximum concentration.
- *Bacillus thuringiensis* achieved significant reductions in all citrus nematode viability and reproductivity parameters.

By plant extracts:

- Plant extracts of *Thuja orientalis* and *Thevetia neriifolia* were highly effective in reducing cyst and citrus nematode populations. The counts of cysts, immature and mature stages were significantly reduced.
- Extracts of *Thuja orientalis* yielded higher rates in nematode reduction when compared to *Thevetia neriifolia*.
- Extracts of both plants achieved higher results in nematode reduction when extracted by the solvent ethyl acetate and applied at the rate of 5%.

2. Can resistant rootstocks be applied as citrus nematode control strategy?

- The rootstock Japanese bitter orange (*Poncirus trifoliata*) showed a high level of resistance against citrus nematode, followed by Volkamer lemon (*Citrus volkameriana*). The rootstocks Cleopatra mandarin (*Citrus reticulata*), Rangpur lime (*Citrus limon*) and Sour orange (*Citrus aurantium*) proved to be moderately resistant while Rough lemon (*Citrus jambhiri*) was susceptible.
- Navel orange scions on *Poncirus trifoliata* or *Citrus volkameriana* showed a very high resistance against citrus nematodes and yielded the maximum nematode decreases even when compared to the nematicide Carbofuran at its maximum dose.

3. When is the most suitable application time of nematode control strategies?

- Two seasonal population peaks of the citrus nematodes were observed to occur in citrus trees. The first occurred in April-May, and the second in October-November. It was found that samples collected at a soil depth of 20 cm contain higher populations of citrus nematodes than those exist at a depth of 50 cm throughout the year.

- According to the previously mentioned findings, the most suitable time of applying nematode control strategies is two months before each seasonal population peak (February-March and August-September).

The understanding of the soil ecosystem management is still at a developmental stage. The control strategies applied in the present work provide a general guideline toward maintaining a healthy soil ecosystem as well as minimising the deleterious effect of the cyst and citrus nematodes by enhancing the antiphytopathogenic potential of soils.

The presented study shows that the antiphytopathogenic potential of soils can be increased by application of organic and inorganic amendments. Furthermore, the biological treatment with fungi filtrates and bacteria are effective methods to decrease nematode affection. The most suitable time for nematode control is before nematode population peaks occur.

Another, time independent option to avoid nematode infestation is the use of resistant citrus rootstocks.

Zusammenfassung: Untersuchungen zur Stärkung das antiphytopathologenen Potentials des Bodens gegen die Zystennematode *Heterodera schachtii* und die Zitrusnematode *Tylenchulus semipenetrans*

Das Ziel der vorliegenden Forschungsarbeit waren Untersuchungen zur Förderung des antiphytopathogenen Potentials des Bodens gegenüber den wirtschaftlich bedeutenden pflanzenparasitären Nematoden *Heterodera schachtii* (Zystennematode der Zuckerrübe) in Deutschland und *Tylenchulus semipenetrans* (Nematode des Zitrusbaumes) in Ägypten. Die eingesetzten Maßnahmen dienen insbesondere der Verbesserung der Bodengüte und Bodengesundheit. Feld- und Gewächshausversuche hierzu wurden in den Jahren 2002-2005 durchgeführt.
Drei Fragen standen im Zentrum der Untersuchung:

1. **Inwiefern kann landwirtschaftliches und biologisches Management die Bodengüte und Bodengesundheit bewahren oder verbessern?**

Durch organische und anorganische Nährstoffversorgung:

- Die Zugabe von handelsüblicher Blumenerde führte in allen Applikationsstufen zu einem signifikanten Rückgang sämtlicher Entwicklungsstadien von *Heterodera schachtii* wie auch der Anzahl der Eier pro Zyste.
- Taubenkot bewirkte eine starke Reduktion aller Entwicklungsstadien von *Tylenchulus semipenetrans*. Eine ähnliche Wirkung wurde durch Zugabe von zerdrücktem Knoblauch erreicht.
- Durch Anwendung eines flüssigen mineralischen Volldüngers (NPK mit Spurenelementen) wurde die Anzahl von Zystennematoden und deren Reproduktion verringert.
- Die Applikation von Ammoniumnitrat reduzierte die Anzahl und Fruchtbarkeit von Zitrusnematoden. Der Effekt verstärkte sich mit steigender Dosierung.
- Die Zugabe von Ascorbin- und Salicylsäure bewirkte einen starken Rückgang der Endpopulation von Zitrusnematoden. Die Steigerung der Dosierung der organischen Säuren und die Abnahme der Reproduktionsleistung der Zitrusnematoden standen in einem proportionalen Verhältnis.

Durch biologische Methoden:

- Filtrate der nematophagen Pilze *Arthrobotrys oligospora*, *Fusarium equiseti*, *Trichoderma harzianum* und *Vertillium lecanii* hatten eine stark nematizide Wirkung auf Zystennematoden. Alle unverdünnten Pilzfiltrate übertrafen die Wirkung des Nematozids Carbofuran und erzielten signifikante Verminderungen des Nematodenbefalls.
- Den stärksten Effekt zur Minderung der Zystennematoden erzielten Filtrate von Fusarium equiseti, gefolgt von *Vertillium lecani*, *Arthrobotrys oligospora* und *Trichoderma harzianum*.
- Gegen Zitrusnematoden wurden Filtrate von *Nematoctonus concurrence* und *Dactylella brochopaga* eingesetzt, deren größte Wirkung ebenfalls bei unverdünnter Applikation beobachtet wurde.
- Die Anwendung des Extraktes von *Bacillus thuringiensis* bewirkte eine signifikante Verringerung der Reproduktions- und Entwicklungsfähigkeit von Zitrusnematoden.

Durch Pflanzenextrakte:

- Pflanzenextrakte von *Thuja orientalis* und *Thevetia neriifolia* erzielten einen starken Rückgang des Nematodenbefalls von Zuckerrüben und Zitronenbäumen. Die Anzahl der Zysten, Eier und Larven der Nematoden verringerte sich signifikant.
- *Thuja orientalis* bewirkte im Vergleich zu Thevetia neriifolia eine stärkere Reduzierung der Nematoden.
- Beide Pflanzenextrakte wiesen die höchste nematizide Wirksamkeit bei Extraktion mit Ethylacetat und 5 %iger Verdünnung auf.

2. Liefert die Verwendung resistenter Wurzelstöcke eine Strategie zur Regulierung von Zitrusnematoden?

- Der Wurzelstock von *Poncirus trifoliata* (Dreiblättrige Orange) zeigte den höchsten Grad an Widerstandskraft gegenüber Zitrusnematoden, gefolgt von *Citrus volkameriana* (Volkamers Zitrone). Die Wurzeln von *Citrus reticulata* (Mandarine), *Citrus limon* (Zitrone) und *Citrus aurantium* (Pomeranze) erwiesen sich gegenüber einem Nematodenbefall als mäßig resistent, während *Citrus jambhiri* (Rauhschalige Zitrone) anfällig war.
- Pfropflinge von *Citrus sinensis* (Navel Orange) auf *Poncirus trifoliata* oder *Citrus volkameriana* entwickelten eine sehr hohe Resistenz gegenüber Zitrusnematoden und

erreichten den maximalen Rückgang an Nematoden, selbst im Vergleich zur Höchstdosis des Nematozids Carbofuran.

3. Welcher Zeitpunkt ist am besten geeignet, Maßnahmen gegen Zitrusnematoden anzuwenden?

- Das Vorkommen der Zitrusnematoden zeigt im jahreszeitlichen Verlauf zwei Populationsspitzen, die erste im April/Mai, die zweite im Oktober/November. Während des gesamten Jahres enthielten die Bodenproben aus einer Tiefe von 20 cm höhere Populationsstärken der Zitrusnematoden als solche aus einer Tiefe von 50 cm.
- Der günstigste Zeitpunkt der Anwendung von Maßnahmen gegen Zitrusnematoden ist zwei Monate vor dem Auftreten der jeweiligen Populationsspitzen im Februar-März bzw. im August-September.

Die Erkenntnisse über das Potential eines Boden-Ökosystem-Managements befinden sich noch in der Entwicklungsphase. Die Strategien zur Nematodenkontrolle der vorliegenden Arbeit bieten allgemeine Richtlinien an, um ein gesundes Bodenökosystem zu erhalten und durch eine Steigerung des antiphytopathologenen Potentials des Bodens den Schaden durch Zysten- und Zitrusnematoden zu minimieren.

Die vorliegende Arbeit zeigt, dass das antiphytopathogene Potential des Bodens durch die Applikation organischer Nährstoffe und anorganischer Nährsalze gesteigert werden kann. Ebenso bewirken die eingesetzten biologischen Methoden eine Reduzierung des Nematodenbefalls der untersuchten Pflanzen. Die Anwendung der Methoden ist am effektivsten bevor die Vermehrung der Nematoden einsetzt.

Die Verwendung resistenter Wurzelstöcke bei Zitrusbäumen ist eine von der Entwicklung der Nematodenpopulation zeitlich unabhängige Möglichkeit zur Vermeidung des Nematodenbefalls.

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7 Appendix

Tab. A.1: Chemical characteristics of the experimental soil

Soil Characteristic	Soil parameter				
	pH	**N_t [mg g^{-1}]**	**P_{CAL} [ppm]**	**K_{CAL} [ppm]**	**Mg [ppm]**
ES	5.85	1.12	173.8	80.6	43.4
$ESPM_1$	5.82	1.25	203.1	137.5	74.3
$ESPM_2$	5.71	1.77	258.1	247.5	142.1
$ESPM_3$	5.87	0.70	155.1	67.2	34.8

ES = Experimental soil, $ESPM_1$ = Experimental soil mixed with 3 % commercial potting mixture, $ESPM_2$ = Experimental soil mixed with 12 % commercial potting mixture.$ESPM_3$ = Experimental soil mixed with 40 % commercial potting mixture

Tab. A.2: The content of carbon (C) and organic soil substances (OSS) of the experimental soil

Soil Characteristic	Soil parameter [%]	
	C_t	**OSS**
ES	0.656	1.130
ES	0.777	1.339
ES	0.674	1.161
ES	0.704	1.213
$ESPM_1$	1.688	2.910
$ESPM_1$	1.069	1.842
$ESPM_1$	1.683	2.901
$ESPM_1$	1.884	3.248
$ESPM_2$	2.834	4.885
$ESPM_2$	2.779	4.790
$ESPM_2$	3.360	5.792
$ESPM_2$	2.778	4.789
$ESPM_3$	5.738	9.892
$ESPM_3$	5.505	9.490
$ESPM_3$	6.366	10.974
$ESPM_3$	5.942	10.224

ES = Experimental soil, $ESPM_1$ = Experimental soil mixed with 3 % commercial potting mixture, $ESPM_2$ = Experimental soil mixed with 12 % commercial potting mixture.$ESPM_3$ = Experimental soil mixed with 40 % commercial potting mixture

Tab. A.3: The water content and maximum water holding capacity of the experimental soil

Soil Characteristic	Soil parameter [%]	
	Water content	**Maximum water holding capacity**
ES	9.49	19.57
ES	8.82	19.74
ES	9.11	19.87
PM	69.76	
PM	69.75	
PM	70.10	

ES = Experimental Soil, PM = Commercial potting mixture

Tab. A.4: Reproductivity of *Heterodera schachtii* infecting sugar beet seedlings as influenced by addition of organic and inorganic fertilisers, biological agents and plant extracts

Treatments	Counts in 100 g soil		Counts in 1 g fresh roots		Eggs cyst^{-1}	Pf Pi^{-1}	Final population	Population reduction [%]
	Cysts	J_2	Immature Stages	Mature females				
Control without treatment	46	1320	140	5	376	8.20	24588	
Control without treatment	61	2416	276	9	412	14.86	44586	
Control without treatment	52	1987	153	6	238	12.23	36702	
Control without treatment	43	1169	96	5	373	7.27	21816	
Control with carbofuran 0.5 g pot^{-1}	37	516	56	1	241	3.32	9954	68.8
Control with carbofuran 0.5 g pot^{-1}	41	617	63	2	312	3.95	11844	62.9
Control with carbofuran 0.5 g pot^{-1}	43	821	61	2	261	5.18	15552	51.3
Control with carbofuran 0.5 g pot^{-1}	46	780	77	3	343	4.96	14868	53.4
Control with carbofuran 1.0 g pot^{-1}	33	671	53	1	267	4.22	12672	60.3
Control with carbofuran 1.0 g pot^{-1}	30	712	51	0	209	4.45	13356	58.2
Control with carbofuran 1.0 g pot^{-1}	36	612	49	1	197	3.89	11664	63.5
Control with carbofuran 1.0 g pot^{-1}	29	623	59	1	243	3.91	11736	63.2
Control with carbofuran 2.0 g pot^{-1}	21	514	50	0	217	3.21	9630	69.8
Control with carbofuran 2.0 g pot^{-1}	30	537	46	0	202	3.40	10206	68.0
Control with carbofuran 2.0 g pot^{-1}	27	604	57	1	271	3.79	11358	64.4
Control with carbofuran 2.0 g pot^{-1}	20	599	49	0	206	3.71	11142	65.1
Commercial potting mixture 3 %	31	647	76	2	320	4.07	12204	61.8
Commercial potting mixture 3 %	37	763	81	3	216	4.80	14400	54.9
Commercial potting mixture 3%	32	814	58	1	286	5.08	15228	52.3
Commercial potting mixture 3%	30	807	69	2	264	5.02	15066	52.8

Tab. A.4 continued

Treatments	Counts in 100 g soil		Counts in 1 g fresh roots		Eggs cyst^{-1}	Pf Pi^{-1}	Final population	Population reduction [%]
	Cysts	J_2	Immature Stages	Mature females				
Commercial potting mixture 12 %	21	634	66	3	272	3.93	11790	63.1
Commercial potting mixture 12 %	23	547	53	1	291	3.42	10260	67.9
Commercial potting mixture 12 %	21	608	48	1	197	3.77	11322	64.5
Commercial potting mixture 12 %	27	560	54	2	280	3.52	10566	66.9
Commercial potting mixture 40 %	19	521	61	2	216	3.24	9720	69.6
Commercial potting mixture 40 %	21	446	52	1	176	2.80	8406	73.7
Commercial potting mixture 40 %	18	631	53	1	183	3.89	11682	63.4
Commercial potting mixture 40 %	13	609	43	0	261	3.73	11196	64.9
OMF 50 %	35	618	67	3	276	3.92	11754	63.2
OMF 50 %	31	817	64	2	312	5.09	15264	52.2
OMF 50 %	30	773	67	2	283	4.82	14454	54.7
OMF 50 %	32	824	72	3	243	5.14	15408	51.7
OMF 100 %	20	516	71	3	203	3.22	9648	69.8
OMF 100 %	27	697	43	1	199	4.34	13032	59.2
OMF 100 %	23	638	49	1	217	3.97	11898	62.7
OMF 100 %	25	673	67	2	229	4.19	12564	60.6
OMF 200 %	25	719	57	1	220	4.46	13392	58.0
OMF 200 %	26	693	74	3	231	4.31	12942	59.5
OMF 200 %	23	716	68	3	263	4.43	13302	58.3
OMF 200 %	30	767	54	2	196	4.78	14346	55.1
***Arthrobotrys oligospora* 50 %**								

Tab. A.4 continued

Treatments	Counts in 100 g Soil		Counts in 1 g fresh roots		Eggs cyst^{-1}	Pf Pi^{-1}	Final population	Population reduction [%]
	Cysts	J_2	Immature Stages	Mature females				
***Arthrobotrys oligospora* 50 %**	29	743	81	3	370	4.63	13896	56.5
***Arthrobotrys oligospora* 50 %**	26	812	78	2	214	5.03	15084	52.7
***Arthrobotrys oligospora* 50 %**	23	816	64	2	286	5.03	15102	52.7
***Arthrobotrys oligospora* 75 %**	25	691	71	2	273	4.30	12888	59.6
***Arthrobotrys oligospora* 75 %**	27	797	67	2	326	4.94	14832	53.5
***Arthrobotrys oligospora* 75 %**	19	683	63	2	286	4.21	12636	60.4
***Arthrobotrys oligospora* 75 %**	23	649	56	1	361	4.03	12096	62.1
***Arthrobotrys oligospora* 100 %**	22	702	58	1	241	4.34	13032	59.2
***Arthrobotrys oligospora* 100 %**	17	683	55	1	266	4.20	12600	60.5
***Arthrobotrys oligospora* 100 %**	21	519	61	2	316	3.24	9720	69.6
***Arthrobotrys oligospora* 100 %**	23	716	53	1	204	4.43	13302	58.3
***Fusarium equiseti* 50 %**	27	673	69	1	306	4.20	12600	60.5
***Fusarium equiseti* 50 %**	23	836	77	4	233	5.15	15462	51.6
***Fusarium equiseti* 50 %**	21	712	70	3	321	4.40	13194	58.7
***Fusarium equiseti* 50 %**	26	699	53	1	315	4.35	13050	59.1
***Fusarium equiseti* 75 %**	22	573	62	3	316	3.57	10710	66.5
***Fusarium equiseti* 75 %**	25	716	58	1	323	4.45	13338	58.2
***Fusarium equiseti* 75 %**	17	836	53	1	263	5.12	15354	51.9
***Fusarium equiseti* 75 %**	23	773	59	2	289	4.78	14328	55.1
***Fusarium equiseti* 100 %**	20	541	53	1	273	3.37	10098	68.4
***Fusarium equiseti* 100 %**	17	716	43	0	301	4.40	13194	58.7

Tab. A.4 continued

Treatments	Counts in 100 g soil		Counts in 1 g fresh roots		Eggs cyst^{-1}	Pf Pi^{-1}	Final population	Population reduction [%]
	Cysts	J_2	Immature Stages	Mature females				
***Fusarium equiseti* 100 %**	15	602	67	3	263	3.70	11106	65.2
***Fusarium equiseti* 100 %**	21	433	48	1	280	2.72	8172	74.4
***Trichoderma harzianum* 50 %**	30	916	114	5	336	5.68	17028	46.7
***Trichoderma harzianum* 50 %**	27	1070	93	3	307	6.58	19746	38.1
***Trichoderma harzianum* 50 %**	31	1093	95	4	293	6.74	20232	36.6
***Trichoderma harzianum* 50 %**	25	904	106	5	270	5.57	16722	47.6
***Trichoderma harzianum* 75 %**	23	764	87	3	281	4.72	14166	55.6
***Trichoderma harzianum* 75 %**	24	694	72	2	304	4.31	12924	59.5
***Trichoderma harzianum* 75 %**	27	783	81	3	271	4.86	14580	54.3
***Trichoderma harzianum* 75 %**	26	841	89	3	243	5.20	15606	51.1
***Trichoderma harzianum* 100 %**	20	612	56	1	214	3.79	11376	64.4
***Trichoderma harzianum* 100 %**	19	763	74	3	169	4.69	14076	55.9
***Trichoderma harzianum* 100 %**	24	778	69	2	286	4.81	14436	54.8
***Trichoderma harzianum* 100 %**	22	617	81	3	262	3.83	11502	64.0
***Verticillium lecanii* 50 %**	27	864	88	4	276	5.35	16038	49.8
***Verticillium lecanii* 50 %**	30	914	74	3	214	5.66	16992	46.8
***Verticillium lecanii* 50 %**	25	817	76	3	319	5.05	15156	52.5
***Verticillium lecanii* 50 %**	23	723	93	4	282	4.48	13428	57.9
***Verticillium lecanii* 75 %**	26	814	69	2	253	5.04	15120	52.6
***Verticillium lecanii* 75 %**	25	761	58	1	246	4.72	14148	55.7
***Verticillium lecanii* 75 %**	22	754	74	3	187	4.66	13968	56.2

Tab. A.4 continued

Treatments	Counts in 100 g soil		Counts in 1 g fresh roots		Eggs cyst^{-1}	Pf Pi^{-1}	Final population	Population reduction [%]
	Cysts	J_2	Immature Stages	Mature females				
***Verticillium lecanii* 75 %**	23	701	73	3	273	4.34	13032	59.2
***Verticillium lecanii* 100 %**	18	523	48	1	203	3.25	9738	69.5
***Verticillium lecanii* 100 %**	23	746	59	1	223	4.61	13842	56.6
***Verticillium lecanii* 100 %**	20	617	69	2	189	3.82	11466	64.1
***Verticillium lecanii* 100 %**	20	793	70	2	214	4.88	14634	54.2
Thevetia neriifolia (e.a.) 1.25 %	36	876	96	3	290	5.47	16416	48.6
Thevetia neriifolia (e.a.) 1.25 %	38	916	102	4	314	5.72	17172	46.2
***Thevetia neriifolia* (e.a.) 1.25 %**	37	761	86	3	267	4.79	14364	55.0
***Thevetia neriifolia* (e.a.) 1.25 %**	42	843	79	3	252	5.31	15930	50.1
***Thevetia neriifolia* (e.a.) 2.5 %**	37	846	83	3	261	5.30	15894	50.2
***Thevetia neriifolia* (e.a.) 2.5 %**	30	853	89	4	212	5.30	15894	50.2
***Thevetia neriifolia* (e.a.) 2.5 %**	33	719	98	5	257	4.51	13536	57.6
***Thevetia neriifolia* (e.a.) 2.5 %**	32	797	82	3	286	4.97	14922	53.3
***Thevetia neriifolia* (e.a.) 5 %**	29	670	68	2	231	4.19	12582	60.6
***Thevetia neriifolia* (e.a.) 5 %**	32	703	79	3	210	4.41	13230	58.6
***Thevetia neriifolia* (e.a.) 5 %**	27	729	81	3	191	4.54	13608	57.4
***Thevetia neriifolia* (e.a.) 5 %**	27	801	71	3	274	4.97	14904	53.3
***Thuja orientalis* (e.a.) 1.25 %**	37	766	86	3	246	4.82	14454	54.7
***Thuja orientalis* (e.a.) 1.25 %**	31	773	81	3	236	4.82	14472	54.7
***Thuja orientalis* (e.a.) 1.25 %**	35	861	91	2	270	5.38	16128	49.5
***Thuja orientalis* (e.a.) 1.25 %**	36	801	74	2	366	5.02	15066	52.8

Tab. A.4 continued

Treatments	Counts in 100 g soil		Counts in 1 g fresh roots		Eggs cyst^{-1}	Pf Pi^{-1}	Final population	Population reduction [%]
	Cysts	J_2	Immature Stages	Mature females				
***Thuja orientalis* (e.a.) 2.5 %**	33	698	78	3	268	4.39	13158	58.8
***Thuja orientalis* (e.a.) 2.5 %**	29	778	83	4	276	4.84	14526	54.5
***Thuja orientalis* (e.a.) 2.5 %**	35	814	62	2	214	5.09	15282	52.1
***Thuja orientalis* (e.a.) 2.5 %**	31	721	81	3	316	4.51	13536	57.6
***Thuja orientalis* (e.a.) 5 %**	31	537	76	3	240	3.41	10224	68.0
***Thuja orientalis* (e.a.) 5 %**	27	733	71	3	267	4.56	13680	57.1
***Thuja orientalis* (e.a.) 5 %**	29	761	68	3	216	4.74	14220	55.5
***Thuja orientalis* (e.a.) 5 %**	25	793	67	2	198	4.91	14724	53.9
***Thevetia neriifolia* (hex.) 1.25 %**	43	986	123	5	283	6.17	18522	42.0
***Thevetia neriifolia* (hex) 1.25 %**	40	814	139	6	314	5.12	15372	51.8
***Thevetia neriifolia* (hex) 1.25 %**	46	1117	105	3	319	6.98	20934	34.4
***Thevetia neriifolia* (hex) 1.25 %**	34	961	112	3	340	5.97	17910	43.9
***Thevetia neriifolia* (hex) 2.5 %**	33	876	116	4	206	5.45	16362	48.7
***Thevetia neriifolia* (hex) 2.5 %**	31	793	124	4	314	4.94	14832	53.5
***Thevetia neriifolia* (hex) 2.5 %**	38	749	103	3	276	4.72	14166	55.6
***Thevetia neriifolia* (hex) 2.5 %**	30	890	98	3	293	5.52	16560	48.1
***Thevetia neriifolia* (hex) 5 %**	30	640	73	3	221	4.02	12060	62.2
***Thevetia neriifolia* (hex) 5 %**	33	719	88	3	293	4.51	13536	57.6
***Thevetia neriifolia* (hex) 5 %**	37	896	68	2	219	5.60	16794	47.4
***Thevetia neriifolia* (hex) 5 %**	29	801	96	3	227	4.98	14940	53.2
***Thuja orientalis* (hex) 1.25 %**	39	926	123	4	263	5.79	17370	45.6

Tab. A.4 continued

Treatments	Counts in 100 g Soil		Counts in 1 g fresh roots		Eggs cyst^{-1}	Pf Pi^{-1}	Final population	Population reduction [%]
	Cysts	J_2	Immature Stages	Mature females				
***Thuja orientalis* (hex) 1.25 %**	42	809	114	3	308	5.11	15318	52.0
***Thuja orientalis* (hex) 1.25 %**	37	841	92	3	276	5.27	15804	50.5
***Thuja orientalis* (hex) 1.25 %**	40	861	89	3	259	5.41	16218	49.2
***Thuja orientalis* (hex) 2.5 %**	30	763	89	3	263	4.76	14274	55.3
***Thuja orientalis* (hex) 2.5 %**	31	859	114	4	231	5.34	16020	49.8
***Thuja orientalis* (hex) 2.5 %**	39	703	97	3	227	4.45	13356	58.2
***Thuja orientalis* (hex) 2.5 %**	29	829	87	3	259	5.15	15444	51.6
***Thuja orientalis* (hex) 5 %**	28	690	64	2	241	4.31	12924	59.5
***Thuja orientalis* (hex) 5 %**	33	831	91	3	220	5.18	15552	51.3
***Thuja orientalis* (hex) 5 %**	26	706	82	3	180	4.39	13176	58.7
***Thuja orientalis* (hex) 5 %**	28	712	69	3	281	4.44	13320	58.3

Tab. A.5: Growth response of sugar beet seedlings infected with *Heterodera schachtii* as influenced by addition of organic and inorganic fertilisers, biological agents and plant extracts

Treatments	Shoot			Root		
	Height [cm]	Fresh weight [g]	Dry weight [g]	Length [cm]	Fresh weight [g]	Dry weight [g]
Control without treatment	11.5	7.15	1.20	61.0	7.26	1.83
Control without treatment	11.5	6.44	1.21	46.0	5.17	1.28
Control without treatment	11	5.74	0.98	50.0	5.43	1.46
Control without treatment	13.5	6.47	1.17	39.0	3.82	0.92
Healthy plants	26.0	24.06	3.44	30.0	28.1	8.11
Healthy plants	22	23.6	2.93	33.0	24.12	6.3
Healthy plants	23	17.95	2.39	41.0	28.83	8.95
Healthy plants	24.5	17.58	2.39	43.0	26.39	6.10
Control with carbofuran 0.5 g pot^{-1}	9	4	0.65	53.0	4.46	1.19
Control with carbofuran 0.5 g pot^{-1}	10	5	0.93	93.0	6.73	1.47
Control with carbofuran 0.5 g pot^{-1}	10	4.12	0.68	59.0	4.24	1.01
Control with carbofuran 0.5 g pot^{-1}	8.5	5.46	0.93	48.0	6.76	1.63
Control with carbofuran 1.0 g pot^{-1}	13	7.45	1.25	52.0	4.38	0.96
Control with carbofuran 1.0 g pot^{-1}	13	7.41	1.13	60.0	7.91	1.96
Control with carbofuran 1.0 g pot^{-1}	13.5	7.38	1.18	75.0	6.69	1.53
Control with carbofuran 1.0 g pot^{-1}	12	8.53	1.53	37.0	8.93	1.95
Control with carbofuran 2.0 g pot^{-1}	13	7.85	1.23	55.0	5.78	1.21
Control with carbofuran 2.0 g pot^{-1}	16	8.14	1.36	60.0	6.92	1.46
Control with carbofuran 2.0 g pot^{-1}	17	10.26	1.79	43.0	7.07	1.67
Control with carbofuran 2.0 g pot^{-1}	16	10.18	1.54	52.0	7.92	1.72

A.5 continued

Treatments	Shoot			Root		
	Height [cm]	Fresh weight [g]	Dry weight [g]	Length [cm]	Fresh weight [g]	Dry weight [g]
Commercial potting mixture 3 %	21	11.44	2.05	39.5	24.77	5.4
Commercial potting mixture 3 %	20	14.02	2.08	38.5	18.8	3.73
Commercial potting mixture 3%	18.5	12.18	2.29	54	16.9	3.14
Commercial potting mixture 3%	17	9.45	1.75	38	16.25	3.48
Commercial potting mixture 12 %	26.5	17.89	3.56	38.5	42.8	8.98
Commercial potting mixture 12 %	26	28.49	4.31	36.5	38.05	10.17
Commercial potting mixture 12 %	22	21.86	3.95	55.5	45	8.71
Commercial potting mixture 12 %	24.5	23.06	3.72	56	34.67	6.98
Commercial potting mixture 40 %	33	40.12	5.86	61	68.16	13.78
Commercial potting mixture 40 %	26	44	7.42	39.5	69.13	15.72
Commercial potting mixture 40 %	34	47.27	7.45	58.5	77.51	17.76
Commercial potting mixture 40 %	29	46.72	8.39	36	55.63	12.18
OMF 50 %	21	14.02	2.48	64	14.34	3.28
OMF 50 %	21	23.91	4.03	65	28.34	9.59
OMF 50 %	20.5	30.83	3.76	40	39.4	7.64
OMF 50 %	20	17.96	3.83	38	18.82	4.51
OMF 100 %	23	25.14	4.01	47	39.61	8.25
OMF 100 %	22.5	29.68	4.36	80	19.97	3.92
OMF 100 %	19	28.49	4.63	41	26.08	6.28
OMF 100 %	19	17.22	3.31	63	34.12	8.32
OMF 200 %	24	24.64	3.63	64	20.7	4.33

A.5 continued

Treatments	Shoot			Root		
	Height [cm]	Fresh weight [g]	Dry weight [g]	Length [cm]	Fresh weight [g]	Dry weight [g]
OMF 200 %	21	25.83	3.93	76	35.21	7.81
OMF 200 %	21	23.12	3.82	67	29.95	7.1
OMF 200 %	22	24.79	4.08	41	20.77	5.33
***Arthrobotrys oligospora* 50 %**	12.5	5.16	1.03	35	5.55	1.34
***Arthrobotrys oligospora* 50 %**	14	6.73	1.19	46	4.1	1.04
***Arthrobotrys oligospora* 50 %**	12	5.91	1.05	45	7.92	1.68
***Arthrobotrys oligospora* 50 %**	13	6.06	1.2	55	10.64	2.36
***Arthrobotrys oligospora* 75 %**	15	9.2	1.31	48	6.75	1.53
***Arthrobotrys oligospora* 75 %**	16.5	8.26	1.43	50	14.33	3.61
***Arthrobotrys oligospora* 75 %**	18	9.77	1.39	92	6.75	1.43
***Arthrobotrys oligospora* 75 %**	14	7.4	1.21	48	10.21	2.27
***Arthrobotrys oligospora* 100 %**	22	15.12	2.63	66	19.81	5.06
***Arthrobotrys oligospora* 100 %**	21	12.27	1.91	32	15.25	3.71
***Arthrobotrys oligospora* 100 %**	21	12.31	2.12	38	17.94	4.21
***Arthrobotrys oligospora* 100 %**	22	10.7	1.57	77	13.31	3.17
***Fusarium equiseti* 50 %**	12	6.67	1.24	40	5.55	1.51
***Fusarium equiseti* 50 %**	11	5.97	1.05	56	6.14	1.44
***Fusarium equiseti* 50 %**	14	6.22	1.17	83	7.81	1.76
***Fusarium equiseti* 50 %**	12	5.97	1.05	55	6.36	1.4
***Fusarium equiseti* 75 %**	16	8.13	1.38	33.5	9.24	1.99
***Fusarium equiseti* 75 %**	13	9.78	1.35	57	12.96	2.98

A.5 continued

Treatments	Shoot			Root		
	Height [cm]	Fresh weight [g]	Dry weight [g]	Length [cm]	Fresh weight [g]	Dry weight [g]
***Fusarium equiseti* 75 %**	12	9.41	1.39	35	13.12	2.88
***Fusarium equiseti* 75 %**	15	6.7	1.01	48	7.25	1.76
***Fusarium equiseti* 100 %**	19	12.66	2.16	47.5	16.33	3.3
***Fusarium equiseti* 100 %**	19.5	11.59	1.67	46	25.75	5.4
***Fusarium equiseti* 100 %**	20	15.57	2.65	25	15.31	3.39
***Fusarium equiseti* 100 %**	18	12.19	1.89	54	12.69	2.48
***Trichoderma harzianum* 50 %**	12	6.98	1.25	61	7.09	1.78
***Trichoderma harzianum* 50 %**	10	6.9	1.2	53	7.68	1.8
***Trichoderma harzianum* 50 %**	11.5	6.75	1.31	41	8.26	1.99
***Trichoderma harzianum* 50 %**	14	6.42	1.06	33	10.69	2.43
***Trichoderma harzianum* 75 %**	15	7.91	1.64	41	4.51	0.94
***Trichoderma harzianum* 75 %**	13.5	7.95	1.39	35	7.13	1.55
***Trichoderma harzianum* 75 %**	15	9.27	1.53	26	9.36	2.24
***Trichoderma harzianum* 75 %**	12.5	8.09	1.39	81	8.15	1.88
***Trichoderma harzianum* 100 %**	15	11.24	1.99	50	12.7	2.89
***Trichoderma harzianum* 100 %**	17	11.38	2.26	70	13.7	2.58
***Trichoderma harzianum* 100 %**	21	15.89	2.56	30	14.97	3.16
***Trichoderma harzianum* 100 %**	17	13.08	2.53	58	12.24	2.5
***Verticillium lecanii* 50 %**	12	9.84	1.75	38	6.15	1.51
***Verticillium lecanii* 50 %**	11	5.41	0.97	35	7.54	1.8
***Verticillium lecanii* 50 %**	12.5	5.55	0.96	37	5.61	1.23

A.5 continued

Treatments	Shoot			Root		
	Height [cm]	Fresh weight [g]	Dry weight [g]	Length [cm]	Fresh weight [g]	Dry weight [g]
***Verticillium lecanii* 50 %**	10	5.1	0.9	48	5.05	1.1
***Verticillium lecanii* 75 %**	12	7.75	1.27	40	8.06	1.81
***Verticillium lecanii* 75 %**	13	10.65	1.72	42	10.73	2.27
***Verticillium lecanii* 75 %**	14.5	8.37	1.37	45	11	2.53
***Verticillium lecanii* 75 %**	13	6.25	1.11	68	4.45	1.12
***Verticillium lecanii* 100 %**	19	8.37	1.5	12	10.63	2.61
***Verticillium lecanii* 100 %**	18	10.8	2.1	40	9.17	2.12
***Verticillium lecanii* 100 %**	14.5	7.65	1.47	45	9.81	2.61
***Verticillium lecanii* 100 %**	23	20.63	2.81	52	24.81	4.73
***Thevetia neriifolia* (e.a.) 1.25 %**	9	6.86	1.2	45	7.04	1.53
***Thevetia neriifolia* (e.a.) 1.25 %**	8	6.86	1.28	48	7.9	2.38
***Thevetia neriifolia* (e.a.) 1.25 %**	12	6.66	1.12	73	7.16	1.8
***Thevetia neriifolia* (e.a.) 1.25 %**	11.5	6.29	1.19	50	6.17	1.36
***Thevetia neriifolia* (e.a.) 2.5 %**	14	7.48	1.12	50	6.42	1.49
***Thevetia neriifolia* (e.a.) 2.5 %**	10	7.8	1.5	55	9.48	2.13
***Thevetia neriifolia* (e.a.) 2.5 %**	14	6.9	1.34	60	8.55	2.17
***Thevetia neriifolia* (e.a.) 2.5 %**	11	5.19	0.85	57	4.13	1.1
***Thevetia neriifolia* (e.a.) 5 %**	14	12.25	1.93	41	15.38	3.9
***Thevetia neriifolia* (e.a.) 5 %**	16	12.47	2.14	44	15.52	3.84
***Thevetia neriifolia* (e.a.) 5 %**	13	11.14	2.22	48	10.8	2.82
***Thevetia neriifolia* (e.a.) 5 %**	12	9.58	1.77	39	12.53	3.06

A.5 continued

Treatments	Shoot			Root		
	Height [cm]	Fresh weight [g]	Dry weight [g]	Length [cm]	Fresh weight [g]	Dry weight [g]
***Thuja orientalis* (e.a.) 1.25 %**	11	5.63	0.92	33	5.55	1.34
***Thuja orientalis* (e.a.) 1.25 %**	13	6.22	0.98	71	6.97	1.63
***Thuja orientalis* (e.a.) 1.25 %**	11	5.91	1.01	40	8.71	1.93
***Thuja orientalis* (e.a.) 1.25 %**	8.5	6.78	1.28	80	6.96	1.64
***Thuja orientalis* (e.a.) 2.5 %**	11	7.12	1.16	61	5.62	1.33
***Thuja orientalis* (e.a.) 2.5 %**	11.5	8.22	1.52	58	9.64	2.33
***Thuja orientalis* (e.a.) 2.5 %**	11	6.06	1	52	7.92	1.94
***Thuja orientalis* (e.a.) 2.5 %**	10.5	6.39	1.13	45	9.53	2.08
***Thuja orientalis* (e.a.) 5 %**	14	12.03	2.16	41	12.32	2.64
***Thuja orientalis* (e.a.) 5 %**	15	10.73	2	47	16.68	4.05
***Thuja orientalis* (e.a.) 5 %**	14.5	10.91	1.53	37	14.7	3.65
***Thuja orientalis* (e.a.) 5 %**	16	8.72	1.45	52	11.49	2.57
***Thevetia neriifolia* (hex.) 1.25 %**	8.5	5.57	1.01	45	6.25	1.26
***Thevetia neriifolia* (hex) 1.25 %**	9	6.5	1.27	40	6.91	1.58
***Thevetia neriifolia* (hex) 1.25 %**	8.5	6.66	1.18	47	6.1	1.16
***Thevetia neriifolia* (hex) 1.25 %**	10	5.69	1.04	52	6.34	1.45
***Thevetia neriifolia* (hex) 2.5 %**	11.5	4.42	0.88	50	4.14	1.05
***Thevetia neriifolia* (hex) 2.5 %**	8	5.78	1.01	80	7.19	1.62
***Thevetia neriifolia* (hex) 2.5 %**	9	7.1	1.33	58	3.94	1.03
***Thevetia neriifolia* (hex) 2.5 %**	11.5	6.81	1.26	87	7.01	1.62
***Thevetia neriifolia* (hex) 5 %**	14	12.99	2.14	60	12.16	3.1

A.5 continued

Treatments	Shoot			Root		
	Height [cm]	Fresh weight [g]	Dry weight [g]	Length [cm]	Fresh weight [g]	Dry weight [g]
***Thevetia neriifolia* (hex) 5 %**	15.5	12.83	2.21	50	12.28	2.86
***Thevetia neriifolia* (hex) 5 %**	18	14.14	2.66	71	22.55	5.46
***Thevetia neriifolia* (hex) 5 %**	17.5	14.33	2.23	70	18.82	4.27
***Thuja orientalis* (hex) 1.25 %**	11	5.52	0.9	43	4.26	0.96
***Thuja orientalis* (hex) 1.25 %**	8	6.79	1.3	85	6.25	1.31
***Thuja orientalis* (hex) 1.25 %**	9	5.81	0.94	63	5.08	1.18
***Thuja orientalis* (hex) 1.25 %**	10	4.16	0.59	61	4.53	1.15
***Thuja orientalis* (hex) 2.5 %**	12	10.38	1.85	38	11.65	2.59
***Thuja orientalis* (hex) 2.5 %**	12	7.04	1.05	43	10.07	2.24
***Thuja orientalis* (hex) 2.5 %**	11	9	1.27	42	10.02	2.62
***Thuja orientalis* (hex) 2.5 %**	12	7.35	1.34	41	8.12	2.01
***Thuja orientalis* (hex) 5 %**	16.5	13.87	2.28	28	12.55	3.56
***Thuja orientalis* (hex) 5 %**	15	10.45	1.77	39	11.56	2.67
***Thuja orientalis* (hex) 5 %**	14	12.1	1.91	58	11.42	2.66
***Thuja orientalis* (hex) 5 %**	14	8.33	1.66	50	6.96	1.55

Tab. A.6: Reproductivity of *Tylenchulus semipenetrans* infecting citrus seedlings as influenced by application of organic and inorganic materials, biological agents, resistant rootstocks and plant extracts

Treatments	Nematode counts				Nematode fecundity			Population reduction [%]
	Egg masses	Immature Stages	J_2	Pf Pi^{-1}	Eggs $eggmass^{-1}$	% egg production	Eggs g^{-1} root	
Control without treatment	226	539	19800	4.11	177	97.2	2301	
Control without treatment	261	678	23273	4.84	186	117.9	2790	
Control without treatment	209	487	18683	3.88	163	82.7	1956	
Control without treatment	243	452	19610	4.06	173	102.4	2422	
Control with carbofuran 0.5 g pot^{-1}	86	155	5850	1.22	83	17.3	415	71.2
Control with carbofuran 0.5 g pot^{-1}	86	189	6418	1.34	112	23.4	560	68.3
Control with carbofuran 0.5 g pot^{-1}	155	258	7788	1.64	111	41.7	999	61.2
Control with carbofuran 0.5 g pot^{-1}	120	172	6028	1.26	78	22.8	546	70.1
Control with carbofuran 1.0 g pot^{-1}	89	124	4115	0.87	69	14.9	345	79.5
Control with carbofuran 1.0 g pot^{-1}	36	89	3298	0.68	89	7.7	178	83.8
Control with carbofuran 1.0 g pot^{-1}	36	142	5260	1.09	83	7.2	166	74.2
Control with carbofuran 1.0 g pot^{-1}	0	71	3540	0.72	0	0.0	0	82.9
Control with carbofuran 2.0 g pot^{-1}	62	104	3585	0.75	73	11.1	219	82.2
Control with carbofuran 2.0 g pot^{-1}	0	83	2818	0.58	0	0.0	0	86.3
Control with carbofuran 2.0 g pot^{-1}	42	125	3758	0.78	79	8.0	158	81.4
Control with carbofuran 2.0 g pot^{-1}	42	104	3043	0.64	82	8.3	164	84.9
Pigeon excrements 25 g pot^{-1}	113	203	4725	1.01	78	21.4	390	76.1
Pigeon excrements 25 g pot^{-1}	113	226	5105	1.09	70	19.2	350	74.2
Pigeon excrements 25 g pot^{-1}	68	158	4865	1.02	82	13.5	246	75.9
Pigeon excrements 25 g pot^{-1}	68	158	4600	0.97	75	12.3	225	77.1

A.6 continued

Treatments	Nematode counts			Nematode fecundity				Population reduction [%]
	Egg masses	Immature Stages	J_2	Pf Pi^{-1}	Eggs $eggmass^{-1}$	% egg production	Eggs g^{-1} root	
Pigeon excrements 50 g pot^{-1}	116	165	5400	1.14	112	31.5	784	73.1
Pigeon excrements 50 g pot^{-1}	99	198	5753	1.21	106	25.6	636	71.3
Pigeon excrements 50 g pot^{-1}	83	149	4840	1.01	97	19.5	485	76.0
Pigeon excrements 50 g pot^{-1}	83	149	4753	1.00	82	16.5	410	76.4
Pigeon excrements 100 g pot^{-1}	107	160	5050	1.06	97	25.2	582	74.8
Pigeon excrements 100 g pot^{-1}	142	231	5753	1.23	125	43.2	1000	71.0
Pigeon excrements 100 g pot^{-1}	124	213	5650	1.20	118	35.7	826	71.6
Pigeon excrements 100 g pot^{-1}	124	213	5575	1.18	123	37.2	861	72.0
Crushed garlic 25 g pot^{-1}	94	265	5850	1.24	100	22.8	600	70.6
Crushed garlic 25 g pot^{-1}	47	234	6875	1.43	120	13.7	360	66.1
Crushed garlic 25 g pot^{-1}	78	250	8933	1.85	111	21.1	555	56.1
Crushed garlic 25 g pot^{-1}	94	312	10518	2.18	132	30.0	792	48.3
Crushed garlic 50 g pot^{-1}	72	180	6290	1.31	87	15.2	348	69.0
Crushed garlic 50 g pot^{-1}	90	197	6003	1.26	92	20.1	460	70.2
Crushed garlic 50 g pot^{-1}	108	287	8550	1.79	121	31.7	726	57.6
Crushed garlic 50 g pot^{-1}	36	251	7400	1.54	98	8.6	196	63.6
Crushed garlic 100 g pot^{-1}	102	321	12830	2.65	125	31.0	875	37.2
Crushed garlic 100 g pot^{-1}	87	262	10150	2.10	94	20.0	564	50.3
Crushed garlic 100 g pot^{-1}	87	189	11508	2.36	101	21.5	606	44.2
Crushed garlic 100 g pot^{-1}	73	262	10425	2.15	76	13.5	380	49.0
Ammonium nitrate 5 g pot^{-1}	226	508	11830	2.51	173	94.9	2076	40.5

A.6 continued

Treatments	Nematode counts			Nematode fecundity				Population reduction [%]
	Egg masses	Immature Stages	J_2	Pf Pi^{-1}	Eggs $eggmass^{-1}$	% egg production	Eggs g^{-1} root	
Ammonium nitrate 5 g pot^{-1}	207	432	9858	2.10	164	82.5	1804	50.3
Ammonium nitrate 5 g pot^{-1}	132	470	11508	2.42	181	57.9	1267	42.7
Ammonium nitrate 5 g pot^{-1}	169	376	11675	2.44	177	72.8	1593	42.1
Ammonium nitrate 10 g pot^{-1}	147	421	8350	1.78	152	54.5	1064	57.8
Ammonium nitrate 10 g pot^{-1}	147	379	8525	1.81	167	59.8	1169	57.1
Ammonium nitrate 10 g pot^{-1}	126	337	7408	1.57	164	50.4	984	62.7
Ammonium nitrate 10 g pot^{-1}	147	337	7718	1.64	123	44.1	861	61.2
Ammonium nitrate 20 g pot^{-1}	133	422	6708	1.45	93	30.2	558	65.6
Ammonium nitrate 20 g pot^{-1}	156	467	7723	1.67	146	55.2	1022	60.5
Ammonium nitrate 20 g pot^{-1}	89	445	7530	1.61	171	37.0	684	61.8
Ammonium nitrate 20 g pot^{-1}	178	267	7370	1.56	107	46.3	856	63.0
Ascorbic acid 1 g pot^{-1}	113	397	10653	2.23	152	41.9	912	47.1
Ascorbic acid 1 g pot^{-1}	113	491	11015	2.32	143	39.4	858	45.0
Ascorbic acid 1 g pot^{-1}	95	378	10243	2.14	127	29.2	635	49.3
Ascorbic acid 1 g pot^{-1}	76	321	10280	2.14	134	24.6	536	49.4
Ascorbic acid 2 g pot^{-1}	65	349	9655	2.01	104	16.5	312	52.3
Ascorbic acid 2 g pot^{-1}	87	392	10183	2.13	123	26.1	492	49.5
Ascorbic acid 2 g pot^{-1}	87	349	9005	1.89	97	20.6	388	55.3
Ascorbic acid 2 g pot^{-1}	65	327	8850	1.85	114	18.1	342	56.2
Ascorbic acid 4 g pot^{-1}	70	298	10150	2.10	121	20.6	484	50.2
Ascorbic acid 4 g pot^{-1}	53	263	9030	1.87	97	12.4	291	55.7

A.6 continued

Treatments	Nematode counts			Nematode fecundity				Population reduction [%]
	Egg masses	Immature Stages	J_2	Pf Pi^{-1}	Eggs $eggmass^{-1}$	% egg production	Eggs g^{-1} root	
Ascorbic acid 4 g pot^{-1}	35	245	8850	1.83	89	7.6	178	56.8
Ascorbic acid 4 g pot^{-1}	53	245	9413	1.94	103	13.2	309	54.0
Salicylic acid 1 g pot^{-1}	130	465	11503	2.42	142	44.9	994	42.7
Salicylic acid 1 g pot^{-1}	111	483	11830	2.48	147	39.8	882	41.2
Salicylic acid 1 g pot^{-1}	149	576	12800	2.70	186	67.2	1488	35.9
Salicylic acid 1 g pot^{-1}	93	409	10690	2.24	152	34.3	760	47.0
Salicylic acid 2 g pot^{-1}	146	438	10838	2.28	126	44.7	882	45.9
Salicylic acid 2 g pot^{-1}	167	521	11758	2.49	132	53.5	1056	41.1
Salicylic acid 2 g pot^{-1}	83	480	11300	2.37	129	26.2	516	43.8
Salicylic acid 2 g pot^{-1}	83	375	10050	2.10	143	29.0	572	50.2
Salicylic acid 4 g pot^{-1}	105	376	9840	2.06	117	29.7	585	51.1
Salicylic acid 4 g pot^{-1}	84	355	9605	2.01	123	25.0	492	52.4
Salicylic acid 4 g pot^{-1}	84	355	9500	1.99	102	20.7	408	52.9
Salicylic acid 4 g pot^{-1}	42	397	10175	2.12	130	13.2	260	49.7
Amino acids 0.5 ml pot^{-1}	131	283	6040	1.29	81	25.7	486	69.4
Amino acids 0.5 ml pot^{-1}	109	239	4858	1.04	112	29.6	560	75.3
Amino acids 0.5 ml pot^{-1}	65	131	3658	0.77	77	12.2	231	81.8
Amino acids 0.5 ml pot^{-1}	65	174	5308	1.11	93	14.8	279	73.7
Amino acids 1.0 ml pot^{-1}	44	131	3573	0.75	123	13.1	246	82.3
Amino acids 1.0 ml pot^{-1}	175	306	5433	1.18	147	62.4	1176	72.0
Amino acids 1.0 ml pot^{-1}	196	327	6215	1.35	103	49.2	927	68.1

A.6 continued

Treatments	Nematode counts			Pf Pi^{-1}	Nematode fecundity			Population reduction [%]
	Egg masses	Immature Stages	J_2		Eggs eggmass^{-1}	% egg production	Eggs g^{-1} root	
Amino acids 1.0 ml pot^{-1}	87	240	5118	1.09	94	20.0	376	74.2
Amino acids 2.0 ml pot^{-1}	125	374	7540	1.61	133	40.4	798	61.9
Amino acids 2.0 ml pot^{-1}	187	312	7155	1.53	141	64.2	1269	63.7
Amino acids 2.0 ml pot^{-1}	83	270	5785	1.23	112	22.7	448	70.9
Amino acids 2.0 ml pot^{-1}	83	229	5925	1.25	148	29.9	592	70.5
***Dactylella brochopaga* 50 %**	178	410	8253	1.77	171	74.1	1710	58.1
***Dactylella brochopaga* 50 %**	160	374	8515	1.81	114	44.5	1026	57.1
***Dactylella brochopaga* 50 %**	160	446	6975	1.52	183	71.4	1647	64.1
***Dactylella brochopaga* 50 %**	143	374	7530	1.61	157	54.4	1256	61.9
***Dactylella brochopaga* 75 %**	74	221	5925	1.24	103	18.4	412	70.5
***Dactylella brochopaga* 75 %**	55	313	6218	1.32	162	21.7	486	68.8
***Dactylella brochopaga* 75 %**	92	276	4980	1.07	133	29.8	665	74.7
***Dactylella brochopaga* 75 %**	166	350	6208	1.34	129	51.9	1161	68.2
***Dactylella brochopaga* 100 %**	38	95	4280	0.88	146	13.5	292	79.1
***Dactylella brochopaga* 100 %**	57	114	4830	1.00	87	12.1	261	76.3
***Dactylella brochopaga* 100 %**	19	57	3938	0.80	112	5.2	112	81.0
***Dactylella brochopaga* 100 %**	19	57	2593	0.53	92	4.3	92	87.4
***Nematoctonus concurrence* 50 %**	133	282	7533	1.59	143	46.2	1144	62.4
***Nematoctonus concurrence* 50 %**	83	216	7340	1.53	107	21.6	535	63.8
***Nematoctonus concurrence* 50 %**	83	199	6600	1.38	123	24.8	615	67.4
***Nematoctonus concurrence* 50 %**	17	216	6758	1.40	96	3.9	96	66.9

A.6 continued

Treatments	Nematode counts			Nematode fecundity				Population reduction [%]
	Egg masses	Immature Stages	J_2	Pf Pi^{-1}	Eggs $eggmass^{-1}$	% egg production	Eggs g^{-1} root	
***Nematoctonus concurrence* 75 %**	33	117	5350	1.10	91	7.4	182	74.0
***Nematoctonus concurrence* 75 %**	50	117	5483	1.13	78	9.5	234	73.2
***Nematoctonus concurrence* 75 %**	50	150	4283	0.90	123	15.0	369	78.8
***Nematoctonus concurrence* 75 %**	67	183	4808	1.01	101	16.4	404	76.0
***Nematoctonus concurrence* 100 %**	0	56	3533	0.72	0	0.0	0	83.0
***Nematoctonus concurrence* 100 %**	19	75	3840	0.79	71	3.2	71	81.4
***Nematoctonus concurrence* 100 %**	19	37	2743	0.56	83	3.8	83	86.7
***Nematoctonus concurrence* 100 %**	56	112	3458	0.73	78	10.6	234	82.8
***Bacillus thuringiensis* 1.0 g pot^{-1}**	62	206	5325	1.12	112	16.9	336	73.5
***Bacillus thuringiensis* 1.0 g pot^{-1}**	62	227	5663	1.19	93	14.0	279	71.8
***Bacillus thuringiensis* 1.0 g pot^{-1}**	83	144	4725	0.99	76	15.2	304	76.5
***Bacillus thuringiensis* 1.0 g pot^{-1}**	103	144	4790	1.01	80	20.1	400	76.1
***Bacillus thuringiensis* 2.0 g pot^{-1}**	43	107	3695	0.77	73	7.6	146	81.8
***Bacillus thuringiensis* 2.0 g pot^{-1}**	0	107	2803	0.58	0	0.0	0	86.2
***Bacillus thuringiensis* 2.0 g pot^{-1}**	64	150	4530	0.95	79	12.4	237	77.5
***Bacillus thuringiensis* 2.0 g pot^{-1}**	64	129	3878	0.81	86	13.5	258	80.7
***Bacillus thuringiensis* 4.0 g pot^{-1}**	136	253	6605	1.40	123	40.7	861	66.9
***Bacillus thuringiensis* 4.0 g pot^{-1}**	156	331	7528	1.60	134	50.7	1072	62.0
***Bacillus thuringiensis* 4.0 g pot^{-1}**	117	233	7918	1.65	102	28.9	612	60.8
***Bacillus thuringiensis* 4.0 g pot^{-1}**	58	156	7278	1.50	117	16.6	351	64.5
Navel orange on *Poncirus trifoliata*	21	85	2558	0.53	58	3.0	58	87.4

A.6 continued

Treatments	Nematode counts			Pf Pi^{-1}	Nematode fecundity			Population reduction [%]
	Egg masses	Immature Stages	J_2		Eggs $eggmass^{-1}$	% egg production	Eggs g^{-1} root	
Navel orange on *Poncirus trifoliata*	0	43	2243	0.46	0	0.0	0	89.2
Navel orange on *Poncirus trifoliata*	21	106	2538	0.53	63	3.3	63	87.4
Navel orange on *Poncirus trifoliata*	0	21	1865	0.38	0	0.0	0	91.1
Navel orange on *citrus volkameriana*	41	144	3368	0.71	92	9.2	184	83.2
Navel orange on *citrus volkameriana*	41	103	3405	0.71	79	7.9	158	83.2
Navel orange on *citrus volkameriana*	0	103	2780	0.58	0	0.0	0	86.3
Navel orange on *citrus volkameriana*	62	186	3515	0.75	87	13.1	261	82.2
***Thevetia neriifolia* (e.a.) 1.25 %**	128	440	11588	2.43	161	50.2	1127	42.4
***Thevetia neriifolia* (e.a.) 1.25 %**	147	458	11780	2.48	165	58.8	1320	41.3
***Thevetia neriifolia* (e.a.) 1.25 %**	147	403	12015	2.51	153	54.6	1224	40.5
***Thevetia neriifolia* (e.a.) 1.25 %**	128	385	10675	2.24	149	46.5	1043	47.0
***Thevetia neriifolia* (e.a.) 2.5 %**	120	360	9800	2.06	141	41.1	846	51.3
***Thevetia neriifolia* (e.a.) 2.5 %**	100	400	10280	2.16	133	32.3	665	48.9
***Thevetia neriifolia* (e.a.) 2.5 %**	140	420	10768	2.27	173	58.9	1211	46.4
***Thevetia neriifolia* (e.a.) 2.5 %**	120	400	10575	2.22	130	37.9	780	47.5
***Thevetia neriifolia* (e.a.) 5 %**	105	314	8575	1.80	129	32.8	645	57.4
***Thevetia neriifolia* (e.a.) 5 %**	105	314	8780	1.84	131	33.3	655	56.4
***Thevetia neriifolia* (e.a.) 5 %**	84	209	7548	1.57	121	24.6	484	62.9
***Thevetia neriifolia* (e.a.) 5 %**	84	251	8285	1.72	95	19.3	380	59.2
***Thevetia neriifolia* (e.a.) 10 %**	124	332	9043	1.90	134	40.5	804	55.0
***Thevetia neriifolia* (e.a.) 10 %**	145	352	9590	2.02	139	49.0	973	52.2

A.6 continued

Treatments	Nematode counts			Nematode fecundity				Population reduction [%]
	Egg masses	Immature Stages	J_2	Pf Pi^{-1}	Eggs $eggmass^{-1}$	% egg production	Eggs g^{-1} root	
***Thevetia neriifolia* (e.a.) 10 %**	104	311	8543	1.79	147	37.0	735	57.6
***Thevetia neriifolia* (e.a.) 10 %**	124	311	9743	2.04	107	32.4	642	51.8
***Thuja orientalis* (e.a.) 1.25 %**	136	446	10760	2.27	153	50.5	1071	46.3
***Thuja orientalis* (e.a.) 1.25 %**	155	407	10540	2.22	143	53.9	1144	47.4
***Thuja orientalis* (e.a.) 1.25 %**	116	329	10663	2.22	103	29.1	618	47.4
***Thuja orientalis* (e.a.) 1.25 %**	155	485	13158	2.76	149	56.2	1192	34.7
***Thuja orientalis* (e.a.) 2.5 %**	124	351	9540	2.00	97	29.2	582	52.6
***Thuja orientalis* (e.a.) 2.5 %**	145	330	10093	2.11	96	33.7	672	50.0
***Thuja orientalis* (e.a.) 2.5 %**	124	330	12435	2.58	102	30.7	612	39.0
***Thuja orientalis* (e.a.) 2.5 %**	145	289	9300	1.95	123	43.2	861	53.9
***Thuja orientalis* (e.a.) 5 %**	89	223	6760	1.41	78	16.9	312	66.5
***Thuja orientalis* (e.a.) 5 %**	111	178	7173	1.49	86	23.3	430	64.7
***Thuja orientalis* (e.a.) 5 %**	67	156	6290	1.30	76	12.3	228	69.2
***Thuja orientalis* (e.a.) 5 %**	67	111	5905	1.22	79	12.8	237	71.2
***Thuja orientalis* (e.a.) 10 %**	129	193	9180	1.90	104	32.5	624	55.0
***Thuja orientalis* (e.a.) 10 %**	107	171	8823	1.82	94	24.5	470	56.9
***Thuja orientalis* (e.a.) 10 %**	64	150	8540	1.75	91	14.2	273	58.5
***Thuja orientalis* (e.a.) 10 %**	86	150	9008	1.85	83	17.3	332	56.2
***Thevetia neriifolia* (hex.) 1.25 %**	116	382	10543	2.21	143	40.5	1001	47.7
***Thevetia neriifolia* (hex.) 1.25 %**	133	416	12028	2.52	153	49.5	1224	40.4
***Thevetia neriifolia* (hex.) 1.25 %**	150	449	13575	2.83	150	54.6	1350	32.9

A.6 continued

Treatments	Nematode counts			Pf Pi^{-1}	Nematode fecundity			Population reduction [%]
	Egg masses	Immature Stages	J_2		Eggs $eggmass^{-1}$	% egg production	Eggs g^{-1} root	
***Thevetia neriifolia* (hex.) 1.25 %**	133	416	13003	2.71	147	47.6	1176	35.8
***Thevetia neriifolia* (hex.) 2.5 %**	123	421	11843	2.48	151	45.1	1057	41.3
***Thevetia neriifolia* (hex.) 2.5 %**	105	351	10425	2.18	163	41.7	978	48.5
***Thevetia neriifolia* (hex.) 2.5 %**	140	456	10860	2.29	156	53.3	1248	45.7
***Thevetia neriifolia* (hex.) 2.5 %**	105	386	11530	2.40	171	43.8	1026	43.1
***Thevetia neriifolia* (hex.) 5 %**	101	342	9958	2.08	132	32.3	660	50.7
***Thevetia neriifolia* (hex.) 5 %**	80	302	9530	1.98	139	27.2	556	53.1
***Thevetia neriifolia* (hex.) 5 %**	101	302	9060	1.89	117	28.6	585	55.2
***Thevetia neriifolia* (hex.) 5 %**	121	362	10148	2.13	147	43.1	882	49.7
***Thevetia neriifolia* (hex.) 10 %**	119	377	10433	2.19	151	43.7	906	48.2
***Thevetia neriifolia* (hex.) 10 %**	159	298	10158	2.12	142	54.8	1136	49.7
***Thevetia neriifolia* (hex.) 10 %**	139	457	11325	2.38	123	41.6	861	43.5
***Thevetia neriifolia* (hex.) 10 %**	119	337	12303	2.55	117	33.9	702	39.6
***Thuja orientalis* (hex.) 1.25 %**	113	371	11540	2.40	132	36.3	924	43.1
***Thuja orientalis* (hex.) 1.25 %**	145	404	13365	2.78	148	52.3	1332	34.1
***Thuja orientalis* (hex.) 1.25 %**	97	436	11785	2.46	131	30.9	786	41.7
***Thuja orientalis* (hex.) 1.25 %**	129	468	13043	2.73	146	45.9	1168	35.4
***Thuja orientalis* (hex.) 2.5 %**	115	458	10425	2.20	123	34.3	738	47.9
***Thuja orientalis* (hex.) 2.5 %**	134	363	9780	2.06	102	33.2	714	51.3
***Thuja orientalis* (hex.) 2.5 %**	134	420	10043	2.12	117	38.0	819	49.8
***Thuja orientalis* (hex.) 2.5 %**	115	382	10175	2.13	122	34.0	732	49.5

A.6 continued

Treatments	Nematode counts			Nematode fecundity				Population reduction [%]
	Egg masses	Immature Stages	J_2	Pf Pi^{-1}	Eggs $eggmass^{-1}$	% egg production	Eggs g^{-1} root	
***Thuja orientalis* (hex.) 5 %**	61	266	8040	1.67	113	16.9	339	60.4
***Thuja orientalis* (hex.) 5 %**	102	286	9350	1.95	121	30.1	605	53.9
***Thuja orientalis* (hex.) 5 %**	102	225	7403	1.55	97	24.1	485	63.4
***Thuja orientalis* (hex.) 5 %**	143	184	7175	1.50	88	30.6	616	64.5
***Thuja orientalis* (hex.) 10 %**	134	268	8785	1.84	121	39.4	726	56.5
***Thuja orientalis* (hex.) 10 %**	134	290	9308	1.95	125	40.7	750	53.9
***Thuja orientalis* (hex.) 10 %**	112	335	7845	1.66	103	27.9	515	60.7
***Thuja orientalis* (hex.) 10 %**	89	268	7325	1.54	196	42.5	784	63.6

Tab. A.7: Growth response of citrus seedlings infected with *Tylenchulus semipenetrans* as influenced by application of organic and inorganic materials, biological agents, resistant rootstocks and plant extracts

Treatments	Shoot			Root		
	Height [cm]	Fresh weight [g]	Dry weight [g]	Length [cm]	Fresh weight [g]	Dry weight [g]
Control without treatment	48.3	31.7	24.3	22.7	17.3	8.2
Control without treatment	41.6	28.9	19	20.2	16.2	7.6
Control without treatment	46.5	29.4	20.6	22.9	18.7	8.3
Control without treatment	43.2	27.1	19.5	21.6	17.3	9.1
Healthy plants	51	38.4	21	27.3	18.6	8.4
Healthy plants	48.7	36.3	19.7	23.2	21.3	10.3
Healthy plants	51.3	42.6	22.2	26.4	22	11.2
Healthy plants	55.4	45.1	23.6	28.6	23.6	11.7
Control with carbofuran 0.5 g pot^{-1}	48.6	35.6	21.3	22.8	16.7	7.9
Control with carbofuran 0.5 g pot^{-1}	46.4	37.4	23.2	19.23	16.2	8
Control with carbofuran 0.5 g pot^{-1}	51.2	40.2	25.7	18.3	18.7	9.3
Control with carbofuran 0.5 g pot^{-1}	52.4	40	26.2	23.7	17.1	8.7
Control with carbofuran 1.0 g pot^{-1}	53.1	42	27.1	22.8	17	9.2
Control with carbofuran 1.0 g pot^{-1}	54.3	43.2	27.8	25.6	19.8	9.8
Control with carbofuran 1.0 g pot^{-1}	47.6	36.7	23.1	20.7	16.3	7.6
Control with carbofuran 1.0 g pot^{-1}	46.8	35.2	22.8	23.4	17.9	8.9
Control with carbofuran 2.0 g pot^{-1}	52.6	43	27.6	22.6	16.9	8.1
Control with carbofuran 2.0 g pot^{-1}	55.4	44.7	28	27.9	20.3	10.6
Control with carbofuran 2.0 g pot^{-1}	51.2	40.2	25.3	25.9	22.1	11.2
Control with carbofuran 2.0 g pot^{-1}	54.8	41.6	23.1	29.2	23.9	11.9

A.7 continued

Treatments	Shoot			Root		
	Height [cm]	Fresh weight [g]	Dry weight [g]	Length [cm]	Fresh weight [g]	Dry weight [g]
Pigeon excrements 25 g pot^{-1}	61.4	49.3	24.3	31.6	23.3	11.3
Pigeon excrements 25 g pot^{-1}	53.2	47	23.1	25.4	21.3	10.8
Pigeon excrements 25 g pot^{-1}	54.7	47.6	22	27.2	23.5	11.2
Pigeon excrements 25 g pot^{-1}	64.3	53.4	23.7	32.4	22.1	11.3
Pigeon excrements 50 g pot^{-1}	51.2	46.3	21.6	27.2	19	8
Pigeon excrements 50 g pot^{-1}	50.1	48	22.1	25.8	18.2	8.6
Pigeon excrements 50 g pot^{-1}	49.6	41	23.3	23.2	19.7	8.9
Pigeon excrements 50 g pot^{-1}	52.1	43	20.1	26	18.2	8.3
Pigeon excrements 100 g pot^{-1}	47.2	39.6	23.1	21.3	18.3	8.2
Pigeon excrements 100 g pot^{-1}	45.2	37.2	21.2	19.8	17.2	8.1
Pigeon excrements 100 g pot^{-1}	43.7	31.1	19.6	17.1	15.7	6.7
Pigeon excrements 100 g pot^{-1}	51.4	40.3	21.7	24.6	19.9	8.3
Crushed garlic 25 g pot^{-1}	45.4	38.2	21.6	23.2	17.3	8.3
Crushed garlic 25 g pot^{-1}	51	41.3	26.4	21.3	13.2	7.1
Crushed garlic 25 g pot^{-1}	47.3	37.6	23.2	21	14	7.5
Crushed garlic 25 g pot^{-1}	52.7	38.4	24.1	24.9	17.9	8.3
Crushed garlic 50 g pot^{-1}	57	42.1	27.2	25.8	16.9	7.1
Crushed garlic 50 g pot^{-1}	61.1	47.3	29.3	29.1	19.6	9.6
Crushed garlic 50 g pot^{-1}	52.7	41	24.8	23.1	17.3	8.4
Crushed garlic 50 g pot^{-1}	55.6	42.7	23.1	27.7	18	8.9
Crushed garlic 100 g pot^{-1}	43.2	37.6	21.8	21.3	14.2	8

A.7 continued

Treatments	Shoot			Root		
	Height [cm]	Fresh weight [g]	Dry weight [g]	Length [cm]	Fresh weight [g]	Dry weight [g]
Crushed garlic 100 g pot^{-1}	47.6	38.9	23	22.6	15.1	7.6
Crushed garlic 100 g pot^{-1}	53.4	40.1	23.9	24	15	7.1
Crushed garlic 100 g pot^{-1}	49.8	37.9	22.1	22.4	14.7	6.9
Ammonium nitrate 5 g pot^{-1}	52.3	44.3	23.9	23.2	18.4	8.9
Ammonium nitrate 5 g pot^{-1}	58.4	48.6	29.3	25.4	20.1	9.3
Ammonium nitrate 5 g pot^{-1}	50.6	41.3	28.8	21.7	19.6	9
Ammonium nitrate 5 g pot^{-1}	51.6	43.2	28.9	20.9	17.1	8.3
Ammonium nitrate 10 g pot^{-1}	54.1	51.1	30.2	26.6	21.3	10.3
Ammonium nitrate 10 g pot^{-1}	52.3	50.6	27.3	25.6	19.6	9.7
Ammonium nitrate 10 g pot^{-1}	60.3	54.3	29.1	29.4	22.7	11.1
Ammonium nitrate 10 g pot^{-1}	55.2	51.4	27.1	27.3	20.6	9.8
Ammonium nitrate 20 g pot^{-1}	61.3	55.3	30.6	28.3	23.1	11.4
Ammonium nitrate 20 g pot^{-1}	58.7	51.2	28.3	27.1	22.7	10.1
Ammonium nitrate 20 g pot^{-1}	57.3	50.6	27.1	27.9	20.3	10.3
Ammonium nitrate 20 g pot^{-1}	60.7	53.2	24.2	28.3	22.8	11.1
Ascorbic acid 1 g pot^{-1}	47.6	36.3	20.6	23.7	20.3	9.6
Ascorbic acid 1 g pot^{-1}	42.4	34.2	18.8	27.2	21.4	11.3
Ascorbic acid 1 g pot^{-1}	48.3	39.7	21.7	21.7	15.7	8.1
Ascorbic acid 1 g pot^{-1}	57.3	43.2	23.2	26.1	18.2	8.5
Ascorbic acid 2 g pot^{-1}	53.4	41.3	22.1	28.3	19.4	9.8
Ascorbic acid 2 g pot^{-1}	50.3	47.2	25.4	24.1	20.7	11.2

A.7 continued

Treatments	Shoot			Root		
	Height [cm]	Fresh weight [g]	Dry weight [g]	Length [cm]	Fresh weight [g]	Dry weight [g]
Ascorbic acid 2 g pot^{-1}	54.8	41.7	20.5	26.8	22.3	10.3
Ascorbic acid 2 g pot^{-1}	57.6	46.8	25.6	28.7	24.8	11.7
Ascorbic acid 4 g pot^{-1}	41.2	33.1	17.6	20.3	15	6.9
Ascorbic acid 4 g pot^{-1}	53.4	37.6	21.1	23.4	17.3	9.1
Ascorbic acid 4 g pot^{-1}	47.6	31.8	16.3	21.2	16.1	7.6
Ascorbic acid 4 g pot^{-1}	58.1	40.1	21.8	27.6	21.7	11.2
Salicylic acid 1 g pot^{-1}	50.4	40.3	23.1	22.1	18.6	9.7
Salicylic acid 1 g pot^{-1}	53.2	38.9	21.2	27.6	20.1	10.9
Salicylic acid 1 g pot^{-1}	47.3	37.6	19.8	20.2	15.3	8
Salicylic acid 1 g pot^{-1}	50.7	38.8	19	24.3	20.3	9.7
Salicylic acid 2 g pot^{-1}	53.2	41.2	21.7	25.3	20.9	9.6
Salicylic acid 2 g pot^{-1}	51.6	40.7	20.9	27.1	21.1	10.3
Salicylic acid 2 g pot^{-1}	50.7	37.8	19.8	24.1	20.3	9.6
Salicylic acid 2 g pot^{-1}	46.8	36.2	19	25.9	21.1	9.8
Salicylic acid 4 g pot^{-1}	53.7	41.6	22.2	27.8	22	11.4
Salicylic acid 4 g pot^{-1}	55.2	43.2	22.9	29.3	23.2	12.6
Salicylic acid 4 g pot^{-1}	50.2	42.4	21.7	25.7	19.6	9.9
Salicylic acid 4 g pot^{-1}	45.6	37.8	19.7	23.9	18.8	8.7
Amino acids 0.5 ml pot^{-1}	60.7	49.6	26.3	31.2	21.2	11.9
Amino acids 0.5 ml pot^{-1}	63.4	48.3	26.4	32.7	21.8	12.8
Amino acids 0.5 ml pot^{-1}	65.2	54.3	27.2	37.1	22.1	14.9

A.7 continued

Treatments	Shoot			Root		
	Height [cm]	Fresh weight [g]	Dry weight [g]	Length [cm]	Fresh weight [g]	Dry weight [g]
Amino acids 0.5 ml pot^{-1}	58.4	47.4	23.2	28.3	21.9	12.3
Amino acids 1.0 ml pot^{-1}	61.3	50.6	22.1	31.7	25.1	12.9
Amino acids 1.0 ml pot^{-1}	53.2	45.3	21.3	28.1	20.6	10.3
Amino acids 1.0 ml pot^{-1}	51.1	44.2	20.7	23.4	19.9	8.7
Amino acids 1.0 ml pot^{-1}	50.9	46.8	23.6	25.1	21.7	10.3
Amino acids 2.0 ml pot^{-1}	47.6	39.8	21.1	23.7	18.9	9.1
Amino acids 2.0 ml pot^{-1}	50.3	41.2	19.6	27.3	22.3	10.3
Amino acids 2.0 ml pot^{-1}	51.9	43.4	19.9	29.2	24.7	11.7
Amino acids 2.0 ml pot^{-1}	50.8	40.6	17.2	22.7	17.3	8.7
***Dactylella brochopaga* 50 %**	47.6	35.9	18.2	23.4	17.8	8.6
***Dactylella brochopaga* 50 %**	48.4	36.3	19.1	24.8	17.9	7.8
***Dactylella brochopaga* 50 %**	50.8	40.1	21.6	25.3	19.4	8.3
***Dactylella brochopaga* 50 %**	49.3	37.4	19	23.6	16.2	7.9
***Dactylella brochopaga* 75 %**	49.8	36.8	19.6	24.3	17.6	7.9
***Dactylella brochopaga* 75 %**	50.8	38.4	20.9	25.6	18.1	8.5
***Dactylella brochopaga* 75 %**	54.6	41.1	22.3	25.9	17.2	8.1
***Dactylella brochopaga* 75 %**	50.1	37.4	20	26.1	20.7	9.7
***Dactylella brochopaga* 100 %**	51.6	37.4	20.7	25.4	17.6	8.3
***Dactylella brochopaga* 100 %**	50.3	38.1	21.3	26.1	18.3	8.9
***Dactylella brochopaga* 100 %**	52.1	40.7	21.6	25.3	19.8	9.7
***Dactylella brochopaga* 100 %**	55.3	42.4	21.9	28.7	20.4	10.1

A.7 continued

Treatments	Shoot			Root		
	Height [cm]	Fresh weight [g]	Dry weight [g]	Length [cm]	Fresh weight [g]	Dry weight [g]
***Nematoctonus concurrence* 50 %**	47.2	38.7	20.7	22.2	15.1	7.1
***Nematoctonus concurrence* 50 %**	46.3	37.3	19.9	24.9	15.9	7.3
***Nematoctonus concurrence* 50 %**	51.4	41.2	21.7	25.1	17.1	8.7
***Nematoctonus concurrence* 50 %**	50.7	40.9	21.9	24.3	18.3	8.9
***Nematoctonus concurrence* 75 %**	47.6	36.9	19.1	23.8	16.7	7.3
***Nematoctonus concurrence* 75 %**	48.8	37.4	20.1	24.6	16.3	7.9
***Nematoctonus concurrence* 75 %**	53.9	42.4	21.3	27.6	18.1	8.4
***Nematoctonus concurrence* 75 %**	50.4	40.6	21.7	23.7	15.6	7.3
***Nematoctonus concurrence* 100 %**	51.3	38.9	21.3	27.2	18.9	8.5
***Nematoctonus concurrence* 100 %**	53.9	43.2	22.1	27.8	17.9	8.2
***Nematoctonus concurrence* 100 %**	55.8	41.7	21.6	28.3	20.3	9.6
***Nematoctonus concurrence* 100 %**	52.7	40.7	20.8	27.6	17.6	8.1
***Bacillus thuringiensis* 1.0 g pot^{-1}**	54.1	41.9	23.1	25.6	20.3	9.3
***Bacillus thuringiensis* 1.0 g pot^{-1}**	55.7	42.3	23.9	24.7	19.9	8.4
***Bacillus thuringiensis* 1.0 g pot^{-1}**	57.2	44.3	27.6	26.8	20.3	9.6
***Bacillus thuringiensis* 1.0 g pot^{-1}**	58.3	46.4	27	27.1	22	10.1
***Bacillus thuringiensis* 2.0 g pot^{-1}**	60.3	51	28	29.2	21.3	10.3
***Bacillus thuringiensis* 2.0 g pot^{-1}**	62.4	53.2	29.1	31.6	22.6	11.1
***Bacillus thuringiensis* 2.0 g pot^{-1}**	57.2	46.1	27.3	27.2	20.6	10
***Bacillus thuringiensis* 2.0 g pot^{-1}**	58.3	47.6	25.2	26.3	21.4	10.4
***Bacillus thuringiensis* 4.0 g pot^{-1}**	53.6	39.3	22.3	23.7	20.3	9.8

A.7 continued

Treatments	Shoot			Root		
	Height [cm]	Fresh weight [g]	Dry weight [g]	Length [cm]	Fresh weight [g]	Dry weight [g]
***Bacillus thuringiensis* 4.0 g pot^{-1}**	50.3	38.2	21.7	22.3	19.9	8.6
***Bacillus thuringiensis* 4.0 g pot^{-1}**	48.6	41.6	22.9	20.1	18.4	8.1
***Bacillus thuringiensis* 4.0 g pot^{-1}**	50.7	39.4	21	23.1	19.2	9
Navel orange on *Poncirus trifoliata*	51.6	40.2	21	23.1	21.7	10.2
Navel orange on *Poncirus trifoliata*	55.3	43.3	22.6	29.3	22.3	10.4
Navel orange on *Poncirus trifoliata*	52.2	42.7	22.3	27.1	20.8	9.5
Navel orange on *Poncirus trifoliata*	51.2	41.9	22	26.3	20.3	9.3
Navel orange on *citrus volkameriana*	49.6	38.6	21.3	24.7	19.8	8.7
Navel orange on *citrus volkameriana*	50.3	39.2	21.6	24.9	20.6	9.4
Navel orange on *citrus volkameriana*	52.7	43.1	22.9	26.2	21.2	10.3
Navel orange on *citrus volkameriana*	51.2	40.6	21.8	27.1	20.9	9.8
***Thevetia neriifolia* (e.a.) 1.25 %**	47.2	33.2	19.1	20.3	18.6	9.3
***Thevetia neriifolia* (e.a.) 1.25 %**	45.3	32.3	18.7	23.4	17.2	8.6
***Thevetia neriifolia* (e.a.) 1.25 %**	43.7	30.2	17.6	18.6	16.9	7.8
***Thevetia neriifolia* (e.a.) 1.25 %**	49.8	35.9	21.9	24.3	20.6	9.3
***Thevetia neriifolia* (e.a.) 2.5 %**	50.4	38.3	23.3	27.3	21.6	10.1
***Thevetia neriifolia* (e.a.) 2.5 %**	53.1	41.3	23.9	28.6	22.2	10.9
***Thevetia neriifolia* (e.a.) 2.5 %**	47.6	35.8	20.6	22.2	18.9	8.7
***Thevetia neriifolia* (e.a.) 2.5 %**	43.2	33.1	18.9	19.7	17.3	8.1
***Thevetia neriifolia* (e.a.) 5 %**	50.3	39.4	20.3	27.7	21.9	11.2
***Thevetia neriifolia* (e.a.) 5 %**	47.8	37.6	19.8	23.3	19.8	9.3

A.7 continued

Treatments	Shoot			Root		
	Height [cm]	Fresh weight [g]	Dry weight [g]	Length [cm]	Fresh weight [g]	Dry weight [g]
***Thevetia neriifolia* (e.a.) 5 %**	52.7	43.1	22.8	25.7	21.2	10.1
***Thevetia neriifolia* (e.a.) 5 %**	51.3	40.7	20.7	24.6	20.8	9.7
***Thevetia neriifolia* (e.a.) 10 %**	50.3	38.9	23.6	24.3	20.3	9.2
***Thevetia neriifolia* (e.a.) 10 %**	49.8	37.3	20.2	22.2	19.6	8.7
***Thevetia neriifolia* (e.a.) 10 %**	51.6	40.1	21.3	26.1	22.1	10.2
***Thevetia neriifolia* (e.a.) 10 %**	52.7	42.3	23.1	25.2	20.9	9.2
***Thuja orientalis* (e.a.) 1.25 %**	46.1	37.8	20.9	23.2	17.9	8.2
***Thuja orientalis* (e.a.) 1.25 %**	45.8	38.1	21	22.4	18.1	8.7
***Thuja orientalis* (e.a.) 1.25 %**	47.6	37.9	19.8	24.7	19.9	9.1
***Thuja orientalis* (e.a.) 1.25 %**	50.7	41.7	22.3	26.1	21.6	9.8
***Thuja orientalis* (e.a.) 2.5 %**	49.1	42.1	22.5	23.9	18.6	8.6
***Thuja orientalis* (e.a.) 2.5 %**	58.1	43.1	22.9	27.1	22.7	10.2
***Thuja orientalis* (e.a.) 2.5 %**	53.7	43.7	23	25.8	21.4	9.9
***Thuja orientalis* (e.a.) 2.5 %**	47.6	38.2	21.1	23.9	19.9	9.1
***Thuja orientalis* (e.a.) 5 %**	52.1	42.3	22.3	24.7	20.3	9.3
***Thuja orientalis* (e.a.) 5 %**	58.4	41.2	21.8	27.1	23.7	11.6
***Thuja orientalis* (e.a.) 5 %**	53.2	43.7	22.8	25.2	22.1	10.6
***Thuja orientalis* (e.a.) 5 %**	57.6	46.7	24	27.2	22.9	11.2
***Thuja orientalis* (e.a.) 10 %**	51.7	41.7	21.7	24.3	20.3	9.2
***Thuja orientalis* (e.a.) 10 %**	49.8	38.1	21.9	24.1	19.9	8.7
***Thuja orientalis* (e.a.) 10 %**	58.1	43.9	22.9	27.3	23.8	11.3

A.7 continued

Treatments	Shoot			Root		
	Height [cm]	Fresh weight [g]	Dry weight [g]	Length [cm]	Fresh weight [g]	Dry weight [g]
***Thuja orientalis* (e.a.) 10 %**	50.7	42.9	22.6	25.1	21.7	10.1
***Thevetia neriifolia* (hex.) 1.25 %**	46.5	32.1	18.7	20.6	16.4	8.1
***Thevetia neriifolia* (hex.) 1.25 %**	48.3	34.6	20	22.8	17.6	8.5
***Thevetia neriifolia* (hex.) 1.25 %**	43.5	27.1	15.7	18.6	15.9	7.9
***Thevetia neriifolia* (hex.) 1.25 %**	45.3	26.6	18	20.8	16.6	8.5
***Thevetia neriifolia* (hex.) 2.5 %**	44.2	28.3	17.8	20.3	16.2	8.2
***Thevetia neriifolia* (hex.) 2.5 %**	48.9	35.6	20.4	23.2	18.9	8.7
***Thevetia neriifolia* (hex.) 2.5 %**	47.4	36.7	19.6	22.9	18.2	8.3
***Thevetia neriifolia* (hex.) 2.5 %**	46.9	30.1	18.1	19.2	16.9	8.5
***Thevetia neriifolia* (hex.) 5 %**	47.3	37.3	20.7	23.3	19.6	9
***Thevetia neriifolia* (hex.) 5 %**	50.2	38.6	20	26.9	20.3	9.8
***Thevetia neriifolia* (hex.) 5 %**	51.9	41.3	22.8	28.1	21.3	10.1
***Thevetia neriifolia* (hex.) 5 %**	47	38.1	21	22.7	19.2	9.3
***Thevetia neriifolia* (hex.) 10 %**	48.9	36.9	20.3	22.1	18.9	8.9
***Thevetia neriifolia* (hex.) 10 %**	49.2	38.6	21.1	25.1	20.1	9.2
***Thevetia neriifolia* (hex.) 10 %**	46.5	31.4	18.6	20.3	17.8	7.8
***Thevetia neriifolia* (hex.) 10 %**	50.9	40.7	22.3	27.6	22.6	11.3
***Thuja orientalis* (hex.) 1.25 %**	48.6	33.2	23.9	21.3	16.7	8.6
***Thuja orientalis* (hex.) 1.25 %**	46.2	30.1	29.7	20.8	16.3	8.4
***Thuja orientalis* (hex.) 1.25 %**	47.9	32.1	25.2	20.7	16.5	8.2
***Thuja orientalis* (hex.) 1.25 %**	44.7	29.3	22.2	19.3	15.1	7.9

A.7 continued

Treatments	Shoot			Root		
	Height [cm]	Fresh weight [g]	Dry weight [g]	Length [cm]	Fresh weight [g]	Dry weight [g]
***Thuja orientalis* (hex.) 2.5 %**	51.3	39.4	26.6	21.9	18.7	8.6
***Thuja orientalis* (hex.) 2.5 %**	52.1	41.3	27	22	21.2	9.7
***Thuja orientalis* (hex.) 2.5 %**	47.3	36.6	22.9	19.6	16.8	8.2
***Thuja orientalis* (hex.) 2.5 %**	50.7	42.3	25.9	21.3	19.7	8.9
***Thuja orientalis* (hex.) 5 %**	48.9	39.3	20.1	23.1	21.8	10.5
***Thuja orientalis* (hex.) 5 %**	50.7	38.8	19.6	26.9	18.3	8.3
***Thuja orientalis* (hex.) 5 %**	52.7	43.3	22	28.2	19	8.5
***Thuja orientalis* (hex.) 5 %**	53.9	43.9	22.6	28.3	22.7	10.7
***Thuja orientalis* (hex.) 10 %**	49.2	38.2	21.7	22.9	21.6	10.3
***Thuja orientalis* (hex.) 10 %**	48.9	38.9	22.9	22.6	20.8	10.2
***Thuja orientalis* (hex.) 10 %**	51.7	42.2	25.3	27.9	23.6	11.2
***Thuja orientalis* (hex.) 10 %**	54.6	44.2	23.6	28.3	23.2	11

Tab. A.8: Reproductivity of *Tylenchulus semipenetrans* as influenced by using different resistant citrus rootstocks

Citrus rootstock	Nematode counts				Nematode fecundity				
	Adult stage	Immature Stages	J_2	Pf Pi^{-1}	Rate of penetration	Eggs $eggmass^{-1}$	% egg production	Eggs g^{-1} root	PRI
Cleopatra mandarin	262	340	15780	3,3	12,0	137	73,6	3699	66,1
Cleopatra mandarin	136	281	13025	2,7	8,3	142	39,5	1988	54,2
Cleopatra mandarin	155	175	15530	3,2	6,6	168	53,5	2688	64,0
Cleopatra mandarin	87	175	7850	1,6	5,2	121	21,7	1089	32,7
Cleopatra mandarin	155	281	14700	3,0	8,7	146	46,5	2336	61,1
Cleopatra mandarin	175	233	16298	3,3	8,1	151	54,1	2718	67,4
Japanese bitter orange	0	36	865	0,2	0,7	0	0,0	0	3,6
Japanese bitter orange	24	95	2295	0,5	2,4	39	1,9	78	9,7
Japanese bitter orange	0	36	1163	0,2	0,7	0	0,0	0	4,8
Japanese bitter orange	12	60	1370	0,3	1,4	35	0,9	35	5,8
Japanese bitter orange	0	36	728	0,2	0,7	0	0,0	0	3,1
Japanese bitter orange	12	24	1043	0,2	0,7	35	0,9	35	4,3
Rangpur lime	100	146	12850	2,6	4,9	125	25,7	1625	52,8
Rangpur lime	108	154	13253	2,7	5,2	139	30,7	1946	54,5
Rangpur lime	100	123	12300	2,5	4,5	112	23,0	1456	50,5
Rangpur lime	92	131	12763	2,6	4,5	123	23,3	1476	52,4
Rangpur lime	77	108	12035	2,4	3,7	103	16,3	1030	49,3
Rangpur lime	85	139	12425	2,5	4,5	112	19,4	1232	51,0
Rough lemon	344	446	26075	5,4	15,8	180	127,0	6660	108,4
Rough lemon	326	512	23035	4,8	16,7	145	96,8	5075	96,3
Rough lemon	233	344	24105	4,9	11,5	162	77,2	4050	99,6

A.8 continued

Citrus rootstock	Nematode counts			Pf Pi^{-1}	Rate of penetration	Nematode fecundity			PRI
	Adult stage	Immature Stages	J_2			Eggs $eggmass^{-1}$	% egg production	Eggs g^{-1} root	
Rough lemon	195	298	20250	4,1	9,9	173	69,3	3633	83,7
Rough lemon	335	493	26510	5,5	16,6	189	129,7	6804	110,3
Rough lemon	242	335	24688	5,1	11,5	200	99,1	5200	101,9
Sour orange	200	252	11450	2,4	9,0	113	46,2	2147	48,0
Sour orange	84	105	10478	2,1	3,8	112	19,3	896	43,0
Sour orange	189	273	12773	2,6	9,2	119	46,1	2142	53,4
Sour orange	105	189	12150	2,5	5,9	126	27,1	1260	50,2
Sour orange	84	231	10775	2,2	6,3	116	20,0	928	44,7
Sour orange	84	147	11150	2,3	4,6	121	20,8	968	45,9
Volkamer lemon	59	186	4600	1,0	4,9	56	6,8	336	19,5
Volkamer lemon	20	39	2285	0,5	1,2	39	1,6	78	9,5
Volkamer lemon	20	49	2233	0,5	1,4	45	1,8	90	9,3
Volkamer lemon	29	49	3008	0,6	1,6	49	3,0	147	12,4
Volkamer lemon	20	39	2340	0,5	1,2	40	1,6	80	9,7
Volkamer lemon	20	49	2753	0,6	1,4	36	1,4	72	11,4

Tab. A.9: Growth response of citrus rootstocks as influenced by the infection of the citrus nematode *Tylenchulus semipenetrans*

Citrus rootstock	Shoot						Root					
	Height [cm]		Fresh weight [g]		Dry weight [g]		Length [cm]		Fresh weight [g]		Dry weight [g]	
	Inoc.	Control	Inoc.	Control	Inoc.	Control	Inoc.	Control	Inoc.	Control	Inoc.	Control
Cleopatra mandarin	48.2	46.8	31.6	30.5	11.2	13.2	11.6	10.3	9.6	9.8	4.1	4.8
Cleopatra mandarin	51.4	56.3	36.4	37.3	13.2	15.1	15.2	16.4	11.7	12.8	5.0	6.5
Cleopatra mandarin	56.1	54.2	34.1	33.4	12.9	14.5	13.4	12.1	9.2	10.6	4.3	4.0
Cleopatra mandarin	38.6	50.9	22.2	32.6	9.4	13.7	9.0	14.6	8.7	9.7	3.7	3.9
Cleopatra mandarin	47.1	54.1	29.6	31.9	9.8	13.5	10.6	13.4	9.0	10.9	4.2	4.1
Cleopatra mandarin	46.9	52.9	30.7	31.0	11.9	13.5	11.0	11.9	9.9	9.9	3.9	4.3
Japanese bitter orange	55.3	56.3	33.6	38.4	16.1	17.6	13.9	17.6	11.6	12.1	4.2	4.8
Japanese bitter orange	54.2	57.4	35.1	41.6	15.9	18.7	14.1	20.3	10.4	13.1	4.9	4.9
Japanese bitter orange	59.6	53.6	46.1	36.2	18.1	15.1	18.8	15.1	13.1	12.5	5.2	5.8
Japanese bitter orange	50.1	48.2	36.4	30.0	15.6	12.7	16.6	12.5	11.9	10.4	4.8	4.9
Japanese bitter orange	50.8	-	31.9	-	12.6	-	12.9	-	13.1	-	5.7	-
Japanese bitter orange	53.7	56	34.7	37.4	13.1	15.0	15.2	16.9	11.4	12.0	4.8	5.0
Rangpur lime	21.6	23.2	18.6	20.1	7.2	8.9	10.2	10.4	8.2	8.7	3.5	3.2
Rangpur lime	18.4	26.4	15.7	22.3	7	9.4	9.7	12.3	7.8	10.8	3.2	4.6
Rangpur lime	26.2	21.3	20.9	16.4	8.9	7.1	11.6	11.1	8.1	9.4	3.6	4.0
Rangpur lime	21	20.9	17.2	15.8	7.2	6.3	10.9	10.8	9.2	8.8	4.1	3.1
Rangpur lime	20.6	25.1	15.6	21.6	6.9	8.9	9.2	12.0	6.7	10.0	2.9	4.2
Rangpur lime	19.5	24.7	15.5	20.8	7.1	8.0	8.6	10.4	5.9	7.9	2.5	2.9
Rough lemon	51	58	35.2	32.5	13.6	15.3	13.2	13.7	8.4	9.6	3.6	5.1
Rough lemon	42.2	54.7	32.4	35.4	13	16.1	12.4	12.3	9.3	10.9	4.3	5.8

A.9 continued

Citrus rootstock	Shoot						Root					
	Height [cm]		Fresh weight [g]		Dry weight [g]		Length [cm]		Fresh weight [g]		Dry weight [g]	
	Inoc.	Control	Inoc.	Control	Inoc.	Control	Inoc.	Control	Inoc.	Control	Inoc.	Control
Rough lemon	51.7	48.6	37.6	27.5	14.1	11.7	12.6	11.4	9.6	9.5	3.8	4.5
Rough lemon	48.6	52.9	30	35.9	11.2	16.4	10.7	12.7	8.7	10.3	3.9	4.8
Rough lemon	51.6	56.4	36.1	38.1	13.2	18.8	13.9	13.9	9.9	10.9	4.4	4.1
Rough lemon	53.4	54.3	32.6	34.7	11.1	15.9	14.6	16.2	9.6	11.4	4.5	4.9
Sour orange	48.4	50.3	31.4	32.7	14.6	13.6	12.1	15.1	10.3	11.1	5.0	5.7
Sour orange	49.2	51.6	34.2	33.2	15.2	14.7	13.6	13.1	11.5	10.3	5.2	4.8
Sour orange	33.8	49.3	25.1	30.4	12.0	13.9	11.4	12.6	9.6	8.9	4.2	3.7
Sour orange	51.7	48.7	35.9	29.6	14.9	12.3	14.2	11.2	12.0	9.1	5.6	4.2
Sour orange	47.1	54.1	29.8	37.4	12.8	14.9	13.1	14.7	9.6	11.6	4.1	4.9
Sour orange	48.4	51.7	30.7	36.7	13.9	15.8	13.3	16.6	10.2	12.9	4.8	5.9
Volkamer lemon	46.0	59.6	37.8	41.2	15.1	18.4	12.7	20.1	8.4	13.6	3.8	5.1
Volkamer lemon	55.2	54.2	35.2	37.7	15.8	16.9	13.6	17.6	9.3	11.3	4.2	5.0
Volkamer lemon	56.4	51.4	33.9	32.1	16.9	15.4	17.1	12.1	12.5	10.4	5.7	4.3
Volkamer lemon	51.2	57.1	35.1	37.6	15.0	17.0	14.2	18.7	10.6	13.8	4.2	6.3
Volkamer lemon	50.7	49.8	32.4	29.4	14.9	14.9	13.6	11.4	9.2	8.9	4.1	3.7
Volkamer lemon	48.0	53.9	30.9	35.9	13.2	16.9	12.9	13.6	8.9	11.0	3.5	5.0

Tab. A.10: Seasonal fluctuation of citrus nematode associated with citrus trees in Qaliubiya governorate from 2002 to 2004 at 20 cm soil depth

		Number of J_2 in 250 g soil									
	Month	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
2002	**June**	3250	2340	2110	1335	3100	2146	1412	916	2104	2075
	July	2760	1846	1040	940	2796	1860	1114	615	1930	2025
	August	2135	1614	1090	910	1814	1621	912	786	1824	2000
	September	2017	1514	1000	960	1730	1566	1000	640	1670	1849
	October	2080	1450	1050	990	1940	1935	1514	790	1914	2070
	November	3595	2416	2240	1845	3567	2490	1796	1340	2890	2590
	December	3140	2000	1940	1514	3300	2540	1350	1000	2760	2210
2003	**January**	2114	2000	1580	2000	2100	1986	1314	1000	2591	2114
	February	2116	1350	1740	1714	2113	1311	967	514	1814	1840
	March	2296	1530	2060	2016	2340	1516	1000	730	2116	2100
	April	3540	2470	2590	3107	2840	1714	1516	1215	3540	3170
	May	3617	2614	2814	3000	3500	2170	2000	1114	3413	3251
	June	2960	1740	1918	2140	2511	1730	1346	890	2540	2416
	July	2290	1187	1514	2340	1912	1690	1201	914	2161	2214
	August	2000	1060	1114	2000	1312	1219	680	746	1721	1816
	September	1906	946	1216	2314	1520	1000	790	614	1700	1514
	October	3100	1980	2000	3590	2740	2560	1740	1901	3390	3511
	November	3940	2312	2540	4000	3100	2760	2100	2340	3740	3860
	December	3560	2119	1350	2801	1960	1814	2194	2001	2980	2000
2004	**January**	1970	1620	1930	1712	1840	1000	980	1000	1930	2310
	February	2514	1520	2000	1811	1960	1214	1261	790	2250	2540
	March	2930	1840	2530	2200	2190	1440	1530	830	2560	2650
	April	2840	2360	2740	1980	2510	1714	1970	1118	2711	2960
	May	2980	2190	2820	1713	2380	1820	1800	1090	2970	2114
	June	1990	1970	2500	1430	2000	1320	1300	1000	2300	2000

Tab. A.11: Seasonal fluctuation of citrus nematode associated with citrus trees in Qaliubiya governorate from 2002 to 2004 at 50 cm soil depth

		Number of J_2 in 250 g soil									
	Month	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
2002	**June**	2870	2140	1840	1910	2900	2170	1640	860	1870	1417
	July	2840	1730	873	970	1960	2460	1200	790	1580	1860
	August	1970	1220	980	800	2300	1430	840	530	1630	1800
	September	1940	1660	890	840	1840	1450	800	730	1370	1590
	October	1960	1240	960	840	2320	1730	1500	530	1710	1930
	November	2960	1970	2320	1560	2690	1960	1830	1460	1340	2460
	December	2470	1730	2100	1270	2190	2350	980	890	1790	2340
2003	**January**	1980	1940	1490	1940	2400	2100	1000	840	1760	1840
	February	1940	1240	1560	1530	1940	1000	1240	780	1630	1490
	March	2110	1360	1980	1960	2310	1612	840	630	1760	1840
	April	2960	1970	2360	2920	2470	1340	1412	1370	2960	3000
	May	3240	2460	1940	2740	3000	2000	1760	1000	2860	2740
	June	2460	1530	1410	1960	1980	1820	1000	680	2400	2000
	July	2140	1000	1310	2230	1460	1530	1000	860	1970	2000
	August	1860	820	980	1690	1000	1120	470	530	730	1320
	September	1640	760	1000	1640	1000	640	700	470	1200	1000
	October	3000	1430	1790	2300	2570	2300	1320	1500	3100	2970
	November	2960	1970	2410	3000	2900	1940	1960	2000	2440	2960
	December	2300	1870	1140	1670	1870	1310	1860	1800	1930	1300
2004	**January**	1740	1412	1640	1620	1612	940	730	1240	1740	2000
	February	2000	1330	1870	2000	1670	1000	980	720	2000	1920
	March	2720	1740	2000	1420	1930	1000	1640	740	2100	2304
	April	2690	2000	2520	2000	1920	1630	1620	1000	2000	2640
	May	2300	2000	2600	1530	2340	1530	1420	1000	2200	2000
	June	2000	1640	1960	1200	1970	1200	1000	640	2100	1400

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