South African plants with nematicidal activity against root-knot nematodes: a review

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Highlights

- Plant-parasitic nematodes have a devastating effect on crop production worldwide
- Infections of plants with *Meloidogyne* nematodes are prevalent
- Nematicidal properties of various plant species have been investigated globally
- In South Africa, only 17 plants have demonstrated efficacy against *Meloidogyne* spp.
- Organic soil amendments derived from plants may protect against nematodes

Abstract

Research on using plants as control agents for plant-parasitic nematodes has received substantial attention due to the ability of plant-derived extracts and compounds to either paralyze or kill nematodes. Nematicidal properties of certain plants have been tested *in vitro* and *in vivo* using glasshouse and field trials, either using powdered meal (plant material) or extracts by incorporating them as soil amendments or as seed treatments. The present review aims to document South African plants used for controlling root-knot nematodes, i.e. *Meloidogyne* spp., summarizing *in vitro* and *in vivo* results of experiments conducted to assess their efficacy. Several databases were mined to obtain information on plant use as organic amendments for controlling *Meloidogyne* infections in South Africa. Inclusion criteria focused on plants as organic amendments and the use of plants against *Meloidogyne* infection. *Meloidogyne incognita* race 1 and race 2 are the most commonly studied nematodes infecting tomatoes, which are highly susceptible to nematode infection. Seventeen plant species were reported to effectively reduce the population density of nematodes in the soil in glasshouse, microplot and field trials. The *in vitro* experiments are generally used as guides for investigating if activity occurs via mortality or egg hatchability. Further research exploring South African plants as control agents for root-knot nematodes, chemicals responsible for activity and the plants' mode of action is warranted.

Keywords: Anthelmintic; Nematicidal; Meloidogyne; Caenorhabditis elegans; South African plants

Contents

1. Introduction and economic impact of plant-parasitic nematodes

According to the Food and Agriculture Organization (FAO, 2017), the world's population is expected to grow to almost 10 billion by 2050, with increasing demand for agricultural commodities. There are several factors influencing current food production, for example climate change and the spread of diseases affecting plants and animals (FAO, 2017). Plant-parasitic nematodes are among the most important economic pests affecting productivity in agriculture, with plant-parasitic nematodes (PPN) representing the most significant pathogens (Nicol et al., 2011). The loss of production due to plant-parasitic nematodes in tropical and sub-tropical climates was estimated to be 14% while in developed countries this figure was 8.8%, which is a serious concern to producers (Sahebani and Hadavi, 2008; Collange et al., 2011). According to the report of Decraemer and Hunt (2006) cited by Jones et al. (2013), more than 4 100 species of plant-parasitic nematodes exist. The most economically important groups of nematodes are the sedentary endoparasites, which include the genera *Heterodera* and *Globodera* (cyst nematodes) and *Meloidogyne* (root-knot nematodes) (Williamson and Gleason, 2003).

There are about 150 species of *Meloidogyne* worldwide and these pathogens have a variety of hosts such as vegetables, grass, fruits and weeds (Nicol et al., 2011). More than 2 000 plant species are susceptible to *Meloidogyne* spp. infestation and this was reported by Agrios (1997) cited in Tranier et al. (2014). *Meloidogyne javanica* (Treub) Chitwood, *Meloidogyne incognita* (Kofoid and White) Chitwood, *Meloidogyne arenaria* (Neal) Chitwood, and *Meloidogyne hapla* Chitwood are among the major agronomically important root-knot nematodes responsible for more than 95% infestation in crops (Sasser et al., 1983; Moens et al., 2009; Khalil, 2014). As reported by Onkendi et al. (2014), 14 *Meloidogyne* spp. have been identified in South Africa and include *M. acronea, M. arenaria, M. chitwoodi, M. enterolobii, M. ethiopica, M. fallax, M. graminicola, M. hapla, M. hispanica, M. incognita, M. javanica, M. kikuyensis, M. partityla* and *M. vandervegtei*. These species affect various crops, including potato, tomato, cotton, soybean, pea, sorghum, banana, lettuce, cucumber, aubergine, pineapple, carrot, tobacco, tea, groundnut, guava, sugarcane, watermelon and numerous crops (Onkendi et al., 2014). *M. incognita* and *M. javanica* species are the most important of the plant-parasitic nematodes, infecting almost all cultivated plants throughout the world and their impact on yield has been estimated to amount to billions of dollars and euros annually (Blaxter et al., 1998; Bleve-Zacheo et al., 2007).

Tomato is one of the most commonly grown vegetables worldwide, and in South Africa it is the second most important crop after potatoes (FAO, 2017). Apart from its rich source of micronutrients, it is also ranked as the first in the world which accounts for 14% of world vegetable production (US$ 1.6 billion market value) (FAO, 2010). According to Seid et al. (2015), reports on *Meloidogyne* spp. infecting tomato plants date back to the end of the 19th century and tomato cultivars have different degrees of susceptibility towards different *Meloidogyne* spp.

1.1 How do nematodes infect plants?

Root-knot nematodes (RKN) are sedentary endoparasites feeding at one site on enlarged and modified cells, and represent the most advanced and successful type of parasitism; they are biotrophic and induce profound changes in the roots of their host as they feed (Andrés et al., 2012). RKN have six distinct life-stages including the egg, first stage juvenile (J1), second stage juvenile (J2), third stage juvenile (J3), fourth stage juvenile (J4) and an adult stage (Agrios, 2005). Juvenile *Meloidogyne* parasites hatch from eggs as vermiform J2, which incorporates the first moult which occurs within the egg. These newly hatched juveniles have a short free-living stage in the rhizosphere of the host plants (Agrios, 2005). They may re-invade the host plants of their parent or migrate through the soil to find a new host (Agrios, 2005). The second stage juveniles invade the root elongation region and migrate in the root until they become sedentary. Wyss et al. (1992) reported that once J2 juveniles are inside the roots, they migrate inter-cellularly through the vascular cylinder by separating cells at the middle lamella. After migration to these tissues, successful parasitism by root-knot nematodes is dependent upon the formation of giant cells. RKN J2 feed from the giant cells for 10-12 days, then cease feeding and moult three times (J2 to J4) over the next two days, and eventually become adults. J3 and J4 stages lack a functional stylet and do not feed (Askary, 2008). Adult females are sedentary for the rest of their life, while males, if present, migrate out of the root to fertilize females (Agrios, 2005). Matured female adults in the roots become gravid and erumpent, and root cells increase in size and number causing the distinctive root gall. As stated by Finley (1981), the life span of an adult female may extend to three months and hundreds of eggs may be produced. Females can continue laying eggs even after harvest of aerial parts of the plants and thus the survival stage between crops is within the eggs. Once embryogenesis is completed and environmental conditions are favourable, a second generation of J2 will hatch from the eggs and invade susceptible plant material (Agrios, 2005).

1.2 Symptoms of nematode infection

Plants infested with RKN include symptoms of chlorosis, wilting, galling of roots and tubers, stunted growth, root lesions and yield loss, and these signs are similar to those of nutrient deficiencies, in particular nitrogen deficiency (Siddiqui et al., 2001; Osei et al., 2011; Nguyen et al., 2018). Root galling is an apparent sign of *Meloidogyne* infection and can easily be diagnosed by farmers (Collange et al., 2011). The induced formed gall in the roots due to nematode infection eventually impedes normal uptake of water and nutrients, and it also facilitates the infection of some soil-borne phytopathogens which may enter the xylem and disrupt the movement of water, thus causing extensive damage to the crop (Jahr et al., 1999; Bird and Kaloshian, 2003).

Chemical control in agricultural production increases yield but has a negative impact on the environment and human beings (Youssef and Eissa, 2014). Due to limitations on the use of chemical pesticides, studies on alternative methods for controlling nematodes have increased, including the identification and implementation of resistant host plants (Bernard et al., 2017). Research on the use of botanicals in managing plant-parasitic nematodes is well documented worldwide. As reported by Prakash and Rao (1997) cited in Singh and Prasad (2014), about 1075 species of higher plants have been reported to possess pesticidal properties against insects, mites, molluscs, birds, rodents, and nematodes of agricultural importance. In South Africa, the use of botanicals in managing plant-parasitic nematodes was initiated by Prof. PW Mashela at the University of Limpopo using the fruits from indigenous *Cucumis* spp. (Meyer, 2017). The purpose of this literature review is to highlight the potential use of plants as organic amendments to control plant-parasitic nematodes compared to chemical nematicides. In addition, South African plants reported to have nematicidal properties since 2002 (up till June 2019) and methods used to assess such activity were assessed. Several databases such as Web of Science, Google Scholar, Science Direct, PubMed, and Scopus were used to source relevant literature. The keywords and filters included and/or in combination were "root-knot nematodes", "controlling strategies", "*Meloidogyne* infection" and "South Africa". Inclusion criteria focused on South African plant use as alternatives to chemical use. This review is anticipated to generate enthusiasm for more studies to explore the rich South African flora for candidates useful in controlling plant-parasitic nematodes.

2. Control methods for plant-parasitic nematodes

Methods for controlling plant-parasitic nematodes that are adopted in most parts of Africa are categorized as chemical, biological or cultural practices. These methods are either practiced singly or in combination to achieve desired results (Onkendi et al., 2014). The main control measures for the management of root-knot nematodes on tomato comprise crop rotation, plant genetic resistance and nematicide application (Talavera et al., 2009). Farmers depend entirely on synthetic pesticides for quick and effective reduction in pest and disease incidence

(Gahukar, 2018). Research on various aspects related to identification of genetic host plant resistance in *Meloidogyne* spp., as well as the agronomic performance of pre-released cultivars that are valuable to producers and the industry is one of the main focus areas in South Africa for managing nematodes infecting plants. The use of chemicals, mode of action and their limitations is described below, followed by a discussion on alternatives including biological control, plants with nematicidal activity as well as methods used in application of organic amendments.

2.1 Chemical use as a management strategy

Nematicides are formulated chemicals that can be applied either as pre-planting chemicals, fumigants or as contact nematicides for nematode control (Strajnar and Sirca, 2011). There are several synthetic nematicides which have been reported to be effective in controlling various species of nematodes including *Meloidogyne* spp., including fumigants and non-fumigants (Chitwood, 2002). Fumigant nematicides were used previously mainly as pre-planting treatments to lower initial population (Pi) density of nematodes due to their high levels of phytotoxicity, and non-fumigants were mainly used as both pre-emergent and post-emergent nematicides (Chitwood, 2002). Fumigant nematicides have low molecular weight and occur as gas or liquids. As they volatize, the gas diffuses through the space between soil while the liquid vaporises at ambient temperatures and moves through air spaces in soil particles. When gas and vapour are applied to the soil, they may penetrate deeply where they can have a broad spectrum of biocidal activity (killing fungi, bacteria or even seeds) and the nematodes living in these spaces are killed (Spurr, 1985). Chemicals that fall under fumigants include chloropicrin, metam-sodium, DMDS, iodomethane, EDB, DBCP, 1,3-D and methyl bromide (Desaeger et al., 2017). In South Africa registered fumigants include 1,3-dichloropropene, ethylene dibromide, 1,3-D plus chloropicrin, metam potassium, metam sodium and methyl bromide/chloropicrin (Jones, 2017). The non-fumigants (carbamates or organophosphates) are formulated as granules while some are available as liquids for spraying on soil or foliage. Non-fumigants move by percolation in soil water, they do not move deeply in soil, have a narrower spectrum of biocidal activity and are non-phytotoxic at suggested use rates and are active at lower dosages (Spurr, 1985). The organophosphate pesticides have been in use since the 1940s while the carbamates were introduced in the 1960s (Casida and Quistad, 1998; Casida and Durkin, 2013). The organophosphates include chemicals such as thionazin, ethoprophos, fenamiphos, fensulfothion, terbuufos, tsazofos, ebufos while the carbamates include aldicarb, aldoxycarb, oxamyl, carbofuran and cloethocarb (Spurr, 1985; Giannakou et al., 2005; Abdel-Rahman et al., 2008; Onkendi et al., 2014). Macrocyclic lactones (avermectins and milbemycins) are other forms of non-fumigants, which are chemical derivatives of soil microorganisms belonging to the genus *Streptomyces* reported in Jayakumar (2009) cited by Khalil (2013). The avermectins include the group of ivermectin, abamectin, doramectin, eprinomectin, and selamectin, which is a family of 16-membered macrocyclic lactones. Abamectin is a potent anthelmintic, insecticide, and miticide used to control pests of humans, animals and crops (Soyuncu et al., 2007).

2.2 Nematicide mode of action

The mode of action for organophosphates and carbamates is through inhibition of the acetylcholinesterase (AChE) enzyme. AChE degrades acetylcholine in the synapse, thus the inhibition of this enzyme allows accumulation of acetylcholine with subsequent excessive stimulation of acetylcholine receptors in associated postsynaptic cells and/ or end organs. The excessive inhibition of AChE (>50-60%) produces signs of toxicity which include autonomic dysfunction, for example excessive secretions of the salivary glands, muscle fasciculation and respiratory depression (Pope, 1999). For instance, fenamiphos as reported by Spurr (1985) is a non-fumigant of the organophosphate class which interferes with normal nerve impulse transmission within the central nervous system of insects. Aldicarb is a form of carbamate that inhibits the nematodes from invading the root system where the chemicals are quickly taken up by the nematode and are subjected to metabolization (Sikora and Hartwig, 1991). The mode of action of avermectins is through blocking the transmittance of electrical activity in nerves and muscle cells, by stimulating the release and binding of gamma-amino butyric acid (GABA) at nerve endings (Campbell et al., 1983; Burkhart, 2000) and disturbing neuromuscular transmission, leading to death (Martin et al., 2002).

2.3 Limitations to using chemical nematicides

The use of chemical control is economically viable for only high-value crops (Tsay et al., 2004). Chemical control in agriculture increases production, however there are drawbacks associated with their application, problems with toxicity to animals and humans, poor target specificity and they are also not cost-effective, especially to resource-poor farmers (McSorley et al., 2008; Collange et al., 2011; Youssef and Eissa, 2014; Ogumo, 2014; Wonang and

Danhap, 2016). The incorrect use of chemicals, such as overdose, frequent applications, and application of pesticides beyond the expiry date and illiteracy of applicators is common in developing and less developed countries (Gahukar, 2014) and this has a negative impact on crop production. The majority of nematicides have been banned due to their adverse environmental effects. Agrawal et al. (2010) stated that when nematicides, fertilizers, herbicides, insecticides and fungicides are applied to cropland, some residues remain in the soil after plant uptake and they may leach into the subsurface waters or they may move to surface water by dissolving in runoff or adsorbing sediment. These residues transform into products that may also contaminate water due to chemical and physical processes and this poses significant risks to the environment and non-target organisms ranging from beneficial soil microorganisms to insects, plants, fish and birds. As reported by Wallace (1973) cited in Olaniyi (2015), on low value and perennial crops, nematicides have been insufficiently effective, too expensive, phytotoxic or they may leave undesirable residues when applied on the growing crops. Pollution of the ozone layer causing atmospheric ozone depletion can also occur (Soler et al., 2016) due to chemical pesticides. Moreover, their continued use can lead to some level of resistance in plant-parasitic nematode species (Onkendi et al., 2014). Methyl bromide was previously used as a soil fumigant, multipurpose pre-plant broad-spectrum to control soil-borne diseases, nematodes, insects and weeds in high-value crops such as tomato, strawberry, cucurbits, nursery crops and flowers (Santos et al., 2006). It was known to provide excellent reduction of soil nematode populations, but its use was largely discontinued in the world after 2005 due to its harmful effects on human beings and to the environment including beneficial organisms (Ibrahim et al., 2006; Renčo et al., 2014). Methyl bromide eliminates mycorrhizae, resulting in poorer plant growth (TNAU, 2015). The World Health Organization estimated over 370 000 deaths each year as a results of deliberate ingestion of pesticides (WHO, 2019).

3. Alternatives to chemical control

The problems with currently-used nematicides have raised the need to find alternative, low input, cost-effective and environmentally-friendly nematode strategies to alleviate the pest problem of root-knot nematodes. Organic amendments are known to have a nematode suppressive effect which depends on many interactions that include the type of compounds released, the dosages, soil characteristics and also the level of nematode population (Collange et al., 2011). As reported by Mashela (2002), farmers in low input agricultural farming systems use organic amendments to suppress plant-parasitic nematodes and to provide essential nutrients to the plants. The inorganic fertilizers containing ammoniacal nitrogen or formulations releasing this form of nitrogen in the soil are effective for suppressing nematode populations (Rodriguez-Kabana, 1986). Urea has been studied as an effective fertilizer and nematicide where the compound is readily converted to ammonia by urease present in the soil (Rodriguez-Kabana, 1986). Youssef and Eissa (2014) reported that chemical fertilizers are costly and cause adverse effects on soil by depleting water holding capacity which happens due to the insufficient uptake of these chemicals by plants.

3.1 Biological control

There are many beneficial microbes that can suppress nematodes and promote the growth of plants and other beneficial microbes (Brinkman et al., 2012). Biopesticides from microorganisms are one of the most effective methods for safe crop-management that work under low to medium disease pressure (Gardener and Fravel, 2002). There are several studies reporting the use of microorganisms for the management of pests including nematodes. As an example, rhizobacterial plant growth promoters have been investigated as biological control agents for plant-parasitic nematodes by several researchers (Siddiqui and Mahmood, 1999; Siddiqui, 2005; Khan et al., 2008). *Pseudomonas aeruginosa*, a bacterial species, was reported to have an impact on tomato growth and it also reduced galling (Shankar et al., 2011). *Streptomyces* species have been found to be good potential agents in improving tomato's defensive ability against RKN infection by improving root zone micro-ecology where *Pseudomonas beteli* bacteria with nematicidal activity increased in the root-zone soil, *Enterobacter asburiae* (growth-promoting bacteria) quantity increased in roots and *Pseudomonas brassicacearum* (plant-pathogenic bacteria) disappeared in roots (Ma et al., 2017). *Bacillus methylotrophicus* strain R2-2 and *Lysobacter antibioticus* strain 13-6 isolated in Yunnan from rhizosphere soils and plant tissues were found to have the highest antagonistic activity against the tomato root-knot nematode *Meloidogyne incognita in vitro* and in greenhouse pot experiments (Zhou et al., 2016). Single isolates of bacterial endophytes isolated from African marigold species *Tagetes erecta* and *Tagetes patula* have shown efficacy in controlling nematodes using endo-root derived bacteria (Sturz and Kimpinski, 2004). There are, however, challenges with using microorganisms as biocontrol agents since they lack consistency due to environmental conditions, soil type and the interactions with resident soil microflora. The microorganism is generally introduced at a concentration considerably lower than that of the microflora (Berry et al., 2009).

3.2 Use of botanicals

Natural plant products known as botanical pesticides or phytochemicals are the most studied organic amendments. They are known to be excellent candidates since they can be developed for use as nematicides themselves, or they can serve as model compounds for the development of chemically synthesized derivatives with enhanced activity or environmental friendliness (Chitwood, 2002). They are also known to be compatible with other types of pesticides (Olaniyi, 2015). Goswami and Vijayalakshmi (1986) conducted studies using pot cultures and *in vitro* studies for testing nematicidal properties of *Andrographis paniculata*, *Calendula officinalis*, *Enhydra fluctuans* and *Solanum khasianum* against the root-knot nematode, *Meloidogyne incognita*. All the plant materials reduced galls and nematode populations in pot trials, with *C. officinalis* and *E. fluctuans* being most effective. Aqueous leaf extracts of *Strychnos nuxvomica* caused 100% mortality of second stage juveniles of *Meloidogyne incognita* at a 2% concentration (Leela et al., 2012). Bawa et al. (2014) conducted a study on neem (*Azadirachta indica*), red-bell pepper (*Capsicum annuum*), ginger (*Zingiber officinale*) and African locust bean (*Parkia biglobosa*) which completely (100%) prevented attack and hatching of *Meloidogyne incognita* eggs and also destroyed 100% the juveniles at 1,000 ppm concentrations (at 10% and above). Natarajan et al. (2006) investigated the effect of the cold water extract of *Tagetes erecta* on soil infested with *M. incognita* and reported that the plant effectively reduced root gall indices of tomato. Gall formation and multiplication of both *M. incognita* and *M. hapla* on tomato roots were significantly reduced by all soil treatments with *Artemisia annua* meal powder and water extract (D'Addabbo et al., 2017).

Neem is one of the most studied plants with components that have attracted global attention for their insecticidal, fungicidal, bactericidal and nematicidal properties (Gajalakshmi and Abbasi, 2004). According to the report of Akhtar and Mahmood (1993), neem has a variety of biologically active ingredients which have different modes of action, making it possible for neem to resist more than 200 species of insects, mites and nematodes. The seed and leaf extracts of neem were found to cause 100% juvenile mortality of the root-knot nematodes and some free-living nematodes (Akhtar and Alam, 1991; Khurma and Singh, 1997; Upadhyay et al., 2003). The plant is also recommended for use against gastrointestinal nematodes and related problems in many parts of the world (Subapriya and Nagini, 2005). Feeding fresh leaves to animals reduced 82% of worm eggs (Chandrawathani et al., 2000). Costa et al. (2008) conducted *in vitro* tests using ethyl acetate and ethanol extracts of *A. indica* on *H. conrtotus* eggs and larvae. Ethanol extracts were effective in inhibiting larval development by 87.11% at 50 mg/mL and inhibited egg hatch by 99.77% at 3.12 mg/mL.

4. Application methods for plant use as soil amendments

Application of plants to reduce the population density of nematodes for crop protection can either be done by applying plants as soil amendments, or using cover crops as biofumigants resistant to the nematodes. The most commonly used method in exploring the effectivity of the plants is via glasshouse experiments. A glasshouse is a controlled environment where temperature is maintained and trials undertaken in these environments can give an indication of activity *in vivo*. After that, it is necessary to confirm the activity under natural conditions such as microplot and field trials where temperature and other environmental conditions are not controlled. Ground leaching technology (GLT) is a method that was developed for use in smallholder farming to mitigate the drawbacks of conventional organic amendments in nematode suppression (Mashela, 2002; Nthangeni and Mashela, 2002). The GLT method requires little material and involves spreading 5 g ground organic amendment per 15 cm radius in a shallow hole around the base of the stem at transplanting. The 5 g quantity amounts to 20 kg.ha^{-1} for 4 000 tomato plants⁄ha (Nthangeni and Mashela, 2002). In this technology, the active ingredients are leached out of the powdered plant material through irrigation water into the rhizosphere of plants with consistent effects on nematode suppression and fertiliser effects on tomato (Mashela and Mphosi, 2001; Mashela, 2002).

5. South African plants with nematicidal activity

The information obtained from the available databases regarding South African plants used in managing plant-parasitic nematodes is shown in Table 1. Based on our literature search, a total of 17 South African plants have been reported to show good activity in reducing the population density of nematodes in glasshouse, microplot and field trials, as well as in *in vitro* tests. *Cucumis myriocarpus* was reported to be one of the most effective and researched plant species in South Africa for controlling *Meloidogyne* infection.

Table 1: South African plants reported to have *in vitro* and *in vivo* nematicidal activity against *Meloidogyne incognita*

Family and plant name	Part used	Experiment type	Active Conc. Tested	Nematodes numbers in: Roots	Nematodes numbers in: Soil	Phytotoxicity	Reference
Brassicaceae *Brassica oleracea*	Leaf	Glasshouse	2g	94%	80%	Unsafe	Mashela et al. (2013)
Solanaceae *Capsicum frutescens* var. Serrano	Fruit	Glasshouse	13.6g	69%		Safe	Thovhakale et al. (2006)
		Microplot	148 kg/ha	59%			Thovhakale et al. (2006)
Fabaceae *Cassia abbreviata*	Leaf	Glasshouse	5g	68-85%		Unsafe	Khosa (2012)
Vitaceae *Cissus cactiformis*	Leaf	Glasshouse	5g	80-94%		Safe	Khosa (2012)
		Microplot	5g	95%			
		Field	5g	83%			
Cucurbitaceae *Cucumis africanus*	Fruit	Greenhouse	3%	845-97%	45-96%%	Safe	Pelinganga (2013)
		Microplot	3%	86-90%	4-5%3%		
		Field	2%	86%	25%		
Cucurbitaceae *Cucumis myriocarpus*	Fruit	Microplot	0.71mt/ha	73-83%	49-68%	Safe	Mashela (2002)
	Fruit	Microplot	3g	78%	81%		Mashela and Pofu (2012)
	Fruit	*In vitro* test	2mg/mL	87-95%		Safe	Muedi (2005)
	Fruit	Greenhouse	3%	97-99%	47-90%	Safe	Pelinganga (2013)
		Microplot	3%	75-80%	26-68%		
		Field	2%	79%	45%		

Table 1: Continued

Family and plant name	Part used	Experiment type	Active Conc. Tested	Nematodes numbers in:		Phytotoxicity	Reference
				Roots	Soil		
C. myriocarpus fruit, *Lippia javanica* leaf and *Ricinus communis* fruit (combined)		Microplot	6g	97-98%		Safe	Mashela et al. (2007)
Solanaceae *Capsicum frutescens*	Fruit	Greenhouse	13.6 g	55-69%			Thovhakale et al. (2006)
Euphorbiaceae *Euphorbia ingens*	Leaf	Glasshouse	3g	73-94%			Khosa (2012)
		Microplot	3g	63%			
		Field	3g	54%			
Convolvulaceae *Ipomoea kituiensis*	Leaf	Glasshouse	3g	77-79%		Unsafe	Khosa (2012)
Verbenaceae *Lippia javanica*	Leaf	Microplot	5 g	79-92 %		Safe	Shimelis et al. (2010)
Capparaceae *Maerua angolensis*	Leaf	Glasshouse	5g	78-88%		Safe	Khosa (2012)
		Microplot	5g	96%			
		Field	5g	85%			
Euphorbiaceae *Ricinus communis* L.	Fruit	Glasshouse	5g	64-74%		Safe	Nthangeni and Mashela (2002)
Leguminosae *Senna petersiana*	Leaf	Glasshouse	5g	74-84%		Unsafe	Khosa (2012)
Euphorbiaceae *Spirostachys africana*	Bark	Microplot	14.8g	37%			Thovhakale et al. (2006)

Table 1: Continued

Family and plant name	**Part used**	**Experiment type**	**Active Conc. Tested**	**Nematodes numbers in:**		**Phytotoxicity**	**Reference**
				Roots	**Soil**		
Apocynaceae *Tabernaemontana elegans*	Leaf	Glasshouse	5g	87-88%		Safe	Khosa (2012)
		Microplot	5g	98%			
		Field	5g	87%			
Amaryllidaceae *Tulbaghia violacea*	Whole part	Glasshouse	4g	64%	30%	Safe	Malungane (2014)
Hyacinthaceae *Urginea sanguinea*	Leaf	Glasshouse	5g	72-77%		Unsafe	Khosa (2012)
Capsicum frutescens (fruits) and *Spirostachys africana* (bark)		Greenhouse	6.8g	54-70%		Safe	Thovhakale et al. (2006)
		Microplot	6.8g	54-59%			

The *in vitro* test done by Muedi (2005) with an activity of 79-87% at 2 mg/mL corroborates results of the glasshouse and field experiments reported by other researchers. The study of Mashela (2002) contrasted with these results as it was found that a minimal reduction of the nematodes in the roots occurred in a microplot experiment. The fruits were reported to have potent chemical compounds with root-knot nematode suppressive properties which are comparable to those of aldicarb and fenamiphos (Mashela et al., 2008). In the study conducted by Mafeo and Mashela (2009), the extracts of ground *C. myriocarpus* fruits inhibited germination of maize, millet, sorghum and onion and were found to be unsuitable in the ground leaching technology system as a pre-emergent bio-nematicide. Two active ingredients of the fruits of *C. africanus* and *C. myriocarpus* belonging to the group of tetracyclic triterpenoids are cucurbitacin B ($C_{32}H_{46}O_9$) which oxidizes to leptodermin ($C_{27}H_{38}O_8$) and cucurbitacin A ($C_{32}H_{46}O_8$) which oxidizes to cucumin ($C_{27}H_{40}O_9$) (Dube and Mashela, 2017) (Figure 1). These phytonematicides were active in killing *Meloidogyne incognita* juveniles *in vitro* and reducing their population density in the field (Dube and Mashela, 2017, 2018; Tseke and Mashela, 2017).

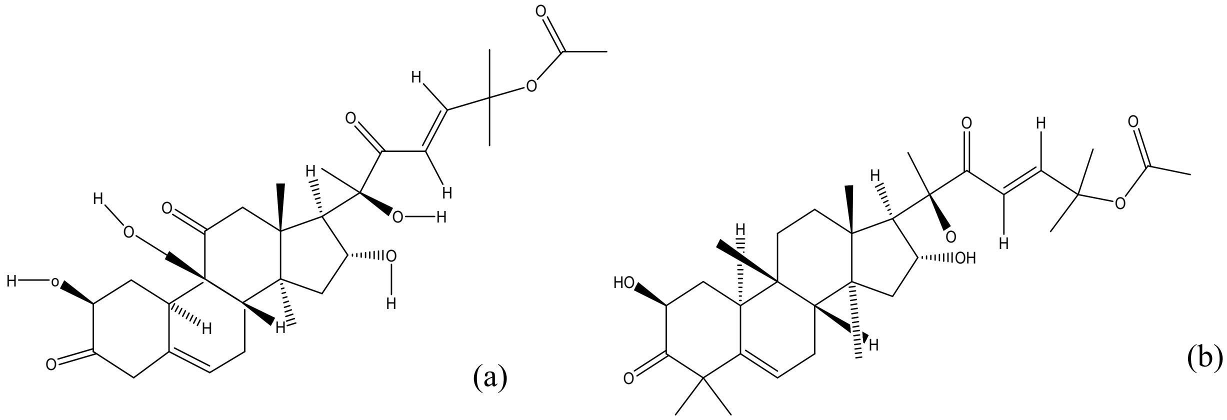

Figure 1: Nematicidal compounds Cucurbitacin A (a) Cucurbitacin B (b) from *Cucumis myriocarpus* and *Cucumis africanus* respectively (Rimington, 1935)

Species belonging to the family Brassicaceae are among the most well-known nematotoxic plants and have been widely investigated in several *in vitro* studies against different phytoparasitic nematode species (Pattison et al., 2006; Argentieri et al., 2011; Wu et al., 2011; Oliveira et al., 2011; Ntalli and Caboni, 2012; Avato et al., 2013; Youssef and Lashein, 2013). Major *Brassica* spp. having biofumigation properties include *B. oleracea* (cole crops: broccoli, cabbage, cauliflower, Brussels sprouts, kale) (Dutta et al., 2019). In the reported study by

Mashela et al. (2013), using the GLT method in a glasshouse, *Brassica oleracea* leaf caused 94% reduction of nematodes in the roots of tomatoes which is supported by several other studies (Lazzeri et al., 2004; Wu et al., 2011; Youssef and Lashein, 2013). Youssef and Lashein (2013) reported that amending the soil with crushed leaves of cabbage reduced the *M. incognita* population in the roots of tomato and also enhanced plant growth. Cruciferous plants belonging to *Brassica* spp. contain glucosinolate compounds. A number of toxic products such as thiocyanate and isothiocyanate are known to be released from these compounds during decomposition (Brown et al., 1991). Isothiocyanates and glucosinolates function as defensive bioactive metabolites against plant pathogens, insects and herbivores (Aires et al., 2009). The pure isothiocyanates demonstrated nematicidal activity against second-stage juveniles of *M. javanica* (Wu et al., 2011). According to Aires et al. (2009), isothiocyanates and glucosinolates can react with biological nucleophiles essential for the nematode, mainly thiol and amine groups of various enzymes which become irreversibly alkylated. The presence of –N=C=S groups and their biological activities induce other types of biological interactions with the nematode hence resulting in high nematicidal activity. The extracts of *B. oleraceae* did not show genotoxic and clastogenic effects in the *in vivo* study (Gonçalves et al., 2012).

Castor (*Ricinus communis* L.) is a member of the Euphorbiaceae family. The plant has been widely studied for its anthelmintic as well as nematicidal activity against helminths infecting animals and humans as well as nematodes parasitizing plants. Adomako and Kwoseh (2013) reported that the extracts of this plant inhibited the J2 larvae of *Meloidogyne* spp. from hatching and caused mortality and Yang et al. (2002) reported mortality of 84.3% *M. incognita* second stage juvenile caused by leaf water extracts. According to Mashela and Nthangeni (2002), the fruits of *R. communis* caused 63% mortality against *M. incognita* juveniles *in vitro* (Table 1). This good activity on *Meloidogyne* spp. was also reported by El-Nagdi and Youssef (2013). Epicatechin was isolated from the methanol leaf extract of *R. communis* and was found to have antiparasitical activity against *Paramphistomum cervi* (a parasite of cattle with a cosmopolitan distribution) (Zahir et al., 2012). The stem and leaf hexane and water extracts had anthelmintic activity against *Caenorhabditis elegans* at high concentrations of 1 and 2 mg/mL, causing 20 and 30% of mortality respectively, and the water extract was not toxic to brine shrimps (McGaw et al., 2007). The seeds of this plant were found to have antifilarial activity against *Brugia malayi* (a parasite causing chronic lymphatic filariasis in human beings) (Shanmugapriya and Ramanathan, 2012). The plant was found to cause toxicosis in a sheep flock (Aslani et al., 2007). In the study conducted by Mongalo et al. (2018), the plant was not toxic at the highest concentration of 0.1 mg/mL to bovine dermis and Vero cell lines. The seeds of *R. communis* contain ricin in quantities of about 1-5% and also have a content of 0.3-0.8% ricinin (Johnson et al., 2005). Ricin is one of the most potent and deadly plant toxins known (Aslani et al., 2007). All parts of the plant contain ricin but the seeds are particularly rich with it and are extremely toxic and are most often associated with clinical toxicosis (Albretsen et al., 2000). The reported toxicity *in vitro* as well as in sheep is most likely due to the presence of ricin.

Other plants were active and exhibited phytotoxic effects on tomato seedlings. In the study conducted by Khosa (2012), the extracts of *C. abbreviata* were found to be active against *Meloidogyne incognita*. However, the plant was found to have phytotoxic or allelopathic effects on the tomato seedlings. According to a survey conducted by Syakalima et al. (2018), the plants have been reported to be used in treating animal diseases by farmers. Mølgaard et al. (2001) reported the leaf, root and bark of *C. abbreviata* to have effects on the cestode *Hymenolepis diminuta*. The plant was also reported to have antimalarial (Kiplagat et al., 2016) and antibacterial activity (Kirabo et al., 2018).

Euphorbia ingens is extremely toxic and its latex can cause severe injuries to the face, eyes, tongue and mouths of humans or animals that come into contact with it (Wink and Van Wyk, 2008). The plant is categorised as poisonous which results in severe dermatitis and blindness (Botha and Penrith, 2008). Ingenol from *E. ingens* was found to help T cells to survive longer against viremia after HIV-1 infection, without exerting cytotoxic effects (Hong et al., 2011). In the study of Khosa (2012), the plant species was reported to have good activity in decreasing the population density of *M. incognita* in *in vivo* trials. However, this plant had some phytotoxic effects on the tomato seedlings. *E. ingens* had suppressive effects against citrus fruits artificially infected with *Penicillium digitatum* (Kena, 2016). The mode of action of most plants is largely unknown, as well as the phytochemicals responsible for the activity. Based on the literature reported, active chemical constituents on plants against *Meloidogyne* spp. are not well documented, for example the mode of action of *R. communis*, *C. abbreviata*, *S. petersiana, Ipomoea kituiensis* is unknown. Toxicity of a plant with nematicidal properties is important, as in the above example of *E. ingens* which is extremely toxic (Wink and Van Wyk, 2008).

6. Is *Caenorhabditis elegans* a suitable test organism?

Caenorhabditis elegans is a free-living nematode naturally found in temperate climate soils belonging to the Order Rhabditida (Blaxter et al., 1998). As stated by Katiki et al. (2011), experimentation with *C. elegans* began in 1960 when researchers were looking for a multicellular organism with a few cells, easy to raise and reproduce for embryonic developmental studies. It was first used in 1965 to study animal development and behaviour by Sydney Brenner (Riddle et al., 1997) and in 1981 was used to screen potential anthelmintic compounds (Simpkin and Coles., 1981). Since then, *C. elegans* has become one of the most studied nematodes in many areas of biology. To work with parasitic nematodes is very difficult since they require a passage through their host for maintenance of their parasitic life-cycle (Holden-Dye and Walker, 2014). *C. elegans* is regarded as the most suitable test organism for preliminary high-throughput *in vitro* screening for compounds with broad spectrum nematicidal activity (Geary and Thompson, 2001). If tested drugs are effective against *C. elegans* cultures at low concentrations, it is reasonable to assume that they may have anthelmintic activity against related nematodes (Thompson et al., 1996). It provides the advantages of a rapid, low cost *in vitro* laboratory method combined with the ability to examine activity of compounds against adult parasitic stages in related nematode species (Katiki et al., 2011). The mode of action of anthelmintic drugs can be evaluated *in vitro* through nematode behaviour, locomotion and reproduction (Katiki et al., 2011). *C. elegans* has been reported to play a major role in defining the mode of action for nematicides and contributing to understanding mechanisms of resistance (Holden-Dye and Walker, 2014). In the development of vaccines against parasitic nematodes, *C. elegans* can be used as a model for the expression of vaccine antigens (Knox, 2012; Murray et al., 2007). However, in some reports poor correlation has been reported against other phytoparasitic nematodes and high levels of broad-spectrum toxins have been identified instead of phytoparsitic nematode-specific compounds (Chitwood, 2002). Fluensulfone is a nematicide of the fluoroalkenyl thioether group and its mode of action was tested using *C. elegans*. The reported results showed that the effective dose required was higher than that with nematicidal activity on *Meloidogyne* spp. but the profile effects of *C. elegans* on motility, egg-hatching and survival were similar to those reported for plant-parasitic nematodes (Kearn et al., 2014). In searching for nematicidal drugs, two or more test organisms should be used for screening as broad spectrum efficacy is of more value in the search for new compounds/drugs. This approach assists in avoiding the limitations that can be brought by using single organisms (Aremu et al., 2012). It is therefore recommended to use this organism as test organism in bioassay guided fractionation of plant extracts for isolation of compounds and also for studying mode of action of drugs on nematodes.

7. Concluding remarks and future recommendations

In conclusion, botanical nematicides are promising tools in *Meloidogyne* spp. control. *In vivo* studies in pots and soil are among the most important methods used in searching for plants that can reduce the population density of nematodes. *In vitro* methods are used as guides for searching for activity and understanding if the extracts inhibit egg hatching or if they act by killing the nematodes after hatching. The use of plants as powdered meal or extracts for application in the field is a challenge since some plants grow in different seasons and their availability in some parts of the country might be difficult. However, exploring more plants, documenting and teaching farmers about them will be an easy method for incorporating them into an overall management system of plant-parasitic nematodes. Studies on active constituents are required for the production of novel nematicidal compounds, and plant secondary metabolites can play a major role in finding lead compounds for chemical synthesis. There are still possible options to study plant-parasitic disease using models such as *Caenorhabditis elegans* which can assist in isolating bioactive constituents and investigating mechanisms of action which can later be tested in the field to confirm activity. Safety of the plants must also be investigated. Plants of South Africa need to be explored for their potential activity against parasites infecting plants, and they can also be investigated further for activity against parasites infecting animals and humans.

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Conflict of interest

The authors declare that they have no conflict of interest.

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