Heliyon 7 (2021) e06306

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Diversity and population distribution of nematodes associated with honeybush (*Cyclopia* spp.) and rooibos (*Aspalathus linearis*) in the Western Cape province of South Africa

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ARTICLE INFO

Keywords:
Colonizers
Maturity indices
Morphological identification
Molecular identification
Soil ecosystem

ABSTRACT

Nematodes are important soil organisms that constitute a key component of the soil ecosystem. A plant-parasitic survey was conducted to identify the diversity of nematodes associated with two endemic tea plants, honeybush (*Cyclopia* spp.) and rooibos (*Aspalathus linearis*) in the Western Cape province of South Africa. A total of 20 farmlands were surveyed and soil samples were collected from the rhizosphere of plants, for nematode isolation and identification based on morphological characters. Confirmation of the species of plant-parasitic nematodes was done using molecular-based tools. Nematodes were classified into various feeding groups based on their colonizer-persister (c-p) values. Plant-feeding nematodes identified from the honeybush tea plants include; *Criconema mutabile*, *Meloidogyne hapla*, *M. javanica*, and *Xiphinema oxycaudatum*, while *Hoplolaimus* sp., *Neodolichorhynchus estherae* and *Pratylechus bolivianus* were pathogenic on the rooibos monocultures. Bacterial and fungal feeders (*Cephalobidae* and *Rhabditidae*) were also abundant and frequently encountered in all samples. The study provides information on the diversity of nematodes associated with the indigenous herbal tea plants of South Africa.

1. Introduction

Nematodes are roundworms that cause significant impact on plant, animal, and human lives and their living ecosystems. They are ubiquitous, diverse, and the most abundant group of multicellular organisms on the earth. Nematodes constitute a key component of the soil food web, occurring at different trophic levels and forming links between plants, bacteria, fungi, and other soil fauna (De Ruiter et al., 1993). Nematode communities in the soil are composed of a variety of trophic and ecological groups, which can be directly linked to key ecosystem functions. Based on their well-classified functional feeding groups, they assume feeding roles such as herbivores, carnivores, and even omnivores. Because of these attributes, soil nematode communities have been regarded as an excellent model system for studying soil health and the impacts of climate change on belowground productivity (Singh and Prasad, 2016).

Nematodes play a very important role as bio-indicators of soil health and multiple roles for regulating plant and animal productivity. They are effective bio-indicators because they are ubiquitous, easy to sample, and well classified into functional feeding groups. Their abundance, species composition, and diversity in a particular ecosystem are important indicators of the stability of the soil environment. Besides, their range of responsiveness to toxins and stresses, such as desiccation, makes them valuable indicators in disturbed systems (Neher, 2001).

Plant-parasitic nematodes are a major threat to agricultural crops worldwide with a global estimate of about $157 billion annually in crop yield losses, being attributed to nematode pests (Abad et al., 2008; Nicol et al., 2011). In many African countries, where food security and poverty remain a huge concern, losses in crop yield due to nematode pests could result in a threat to the source of livelihood of many resource-poor farmers and this could have a significant impact on the economy and foreign exchange earnings of the developing nations.

In South Africa, two indigenous tea crops, *Aspalathus linearis* (Burm.f.) R. Dahlgren (rooibos) and *Cyclopia* spp. (honeybush) are widely cultivated for their known health benefits, among which is their ability to reduce the growth of cancer cells (Marnewick et al., 2005; SARC, 2016). The tea industry in South Africa is currently a thriving business, which has experienced a boost in recent times, due to the high demand for the

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https://doi.org/10.1016/j.heliyon.2021.e06306
Received 12 July 2020; Received in revised form 22 November 2020; Accepted 15 February 2021

Figure 1. Rooibos (A) and honeybush (B) plantations in Western Cape Province of South Africa.

indigenous African herbal tea plants. Both local and international demand for rooibos and its relative honeybush has resulted in intensified cultivation of the tea plants with an increased number of land acreage being extensively cultivated (Department of Agriculture, Forestry and Fisheries, 2011).

Large scale and commercial cultivation of these crops involve practices such as excessive tillage, use of herbicides, and pesticide application. These activities have a potential negative impact on nematode community structures and create spatial changes in nematode assemblage under cultivated soils. Some of the negative environmental implications of intensive cultivation include soil degradation, accumulation of pesticides, diminished availability and quality of water, and compromised soil biodiversity. Soils in agricultural fields are often disturbed and plant residues are not allowed to accumulate on the surface. These and other high input mechanical and chemical management practices cause perturbations that are not congenial to nematode community structure and soil health and can, as a result, contribute significantly to global warming.

The impact of climate change and its threat to life existence has received global attention in recent times. Global warming and climate changes in addition to pathogens and diseases have been identified as the greatest threat to global health in the twenty-first century (Patz et al., 2005). Climate changes may affect the frequency, density, and geographical distribution of parasites and nematodes by directly affecting their infective stages in the environment and the sedentary endo-parasitic forms in plant tissues. Interactions between herbivorous nematodes and plants are also likely to change as a result of climate influence. Therefore, it is necessary to intensify studies focused on the below-ground effect of climate changes on important soil organisms among which nematodes play a key role.

There is a dearth of information on the diversity and distribution of nematodes associated with honeybush and rooibos tea plants. However, there are indications that honeybush is highly susceptible to root-knot nematodes (Hart et al., 2005; Daramola et al., 2020). In the same vein, the lesion nematode, *Pratylenchus bolivianus* Corbett has been reported to be associated with rooibos (Daramola et al., 2018).

Therefore, this study aims to provide information on the diversity of nematodes associated with honeybush and rooibos in South Africa.

2. Material and methods

2.1. Field survey and sampling

A survey for nematodes associated with honeybush and rooibos was conducted between February 2017 and December 2018. Six conventional honeybush farmlands were sampled in Genadendal in the Overberg area of Western Cape Province of South Africa. A field survey was also conducted on fourteen conventional rooibos monocultures in the Cederberg area of the Western Cape (Figure 1). Farmlands were purposively selected based on the accessibility and consent of farmers. Soil samples for the nematology study were collected from the rhizosphere of plants, at a depth of about 20–30 cm into the soil, using a hand trowel. About 25 soil samples were collected per hectare of farmland and bulked. Composite samples were taken from the bulk, bagged, and transported to the laboratory for nematode assay. The number of samples per field varied according to farm size.

2.2. Isolation and morphological identification of nematode species

Nematodes were extracted from soil samples using Cobb's decanting and sieving method (Cobb, 1918), concentrated into 250ml beakers, and poured over a modified Whitehead and Hemming (1965) Extraction Tray set-up. Live nematodes were collected from the extraction tray after a period of 48 h and examined under a stereoscopic microscope to identify the parasitic from the free-living species. Morphological identification of nematodes was done under a compound microscope using the monographs of Heyns (1971); Goodey (1963), and an interactive diagnostic key as a guide. Morphological characters and morphometrics of important plant-parasitic nematodes were measured and light micrographs were taken with the Leica 200 compound microscope, also the free-living nematodes were identified to the family-level, counted, and grouped into their different feeding guilds. The nematodes were counted and assigned to families according to Bongers (1999). Nematode diversity was determined using Shannon's diversity (H′) and Simpson diversity (D) indices (Ferris and Bongers, 2009). Measures of maturity indices (MI) were calculated for the free-living and plant-feeding nematodes.

2.3. DNA extraction and polymerase chain reaction

DNA was extracted from individual plant-parasitic nematodes for molecular identification using a modified method of Nguyen (2007). Second stage infective juvenile (J2) of *Meloidogyne* species and individual single adult female nematodes of other identified plant-parasitic nematodes were handpicked and placed in lysis buffer (500 mM MgCl, 10 mM DTT, 4.5% Tween 20, 0.1% gelatine, and 3 µl proteinase K at 600 µg ml^{-1}). The quantity of lysis buffer (10–30 µl) was varied according to the size of the individual nematodes. The nematodes were then cut into 2–3 parts in the lysis buffer which was placed on the side of an Eppendorf tube. The tubes were kept at -80 °C for a period of 15 min and incubated in a thermocycler at 65 °C for 1 h and then at 95 °C for 15 min.

Polymerase chain reaction (PCR) for the amplification of the DNA products was carried out with KAPA2G™ 40 Robust Hotstart ReadyMix (KAPA Biosystems) using specific sequence-characterized-amplified-region (SCAR) primers for the identification of the *Meloidogyne* species.

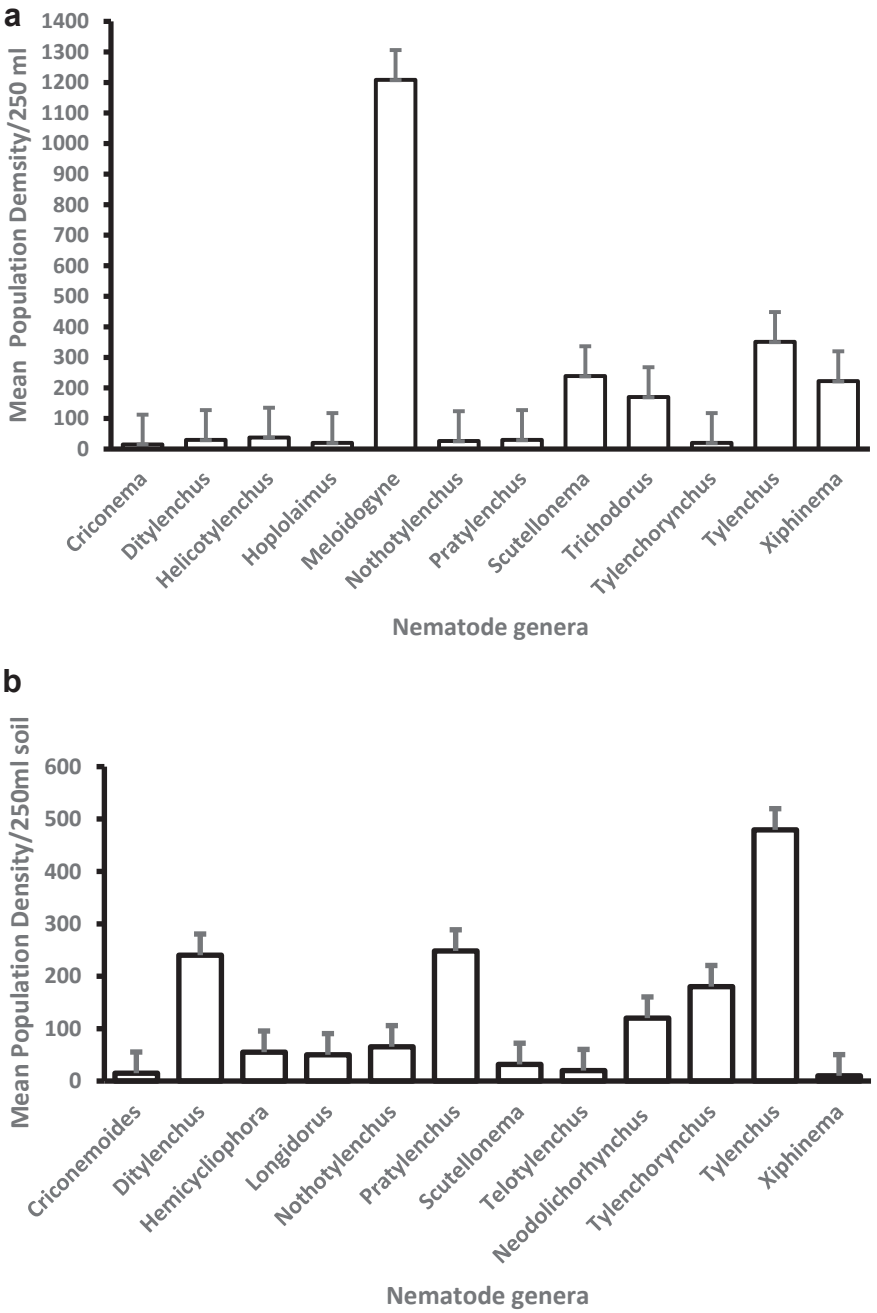

Figure 2. (a) Population density of plant-parasitic nematodes associated with honeybush monocultures in the Western Cape Province of South Africa. (b) Population density of plant-parasitic nematodes associated with rooibos monocultures in the Western Cape Province of South Africa.

Table 1. Families and c-p values of plant-parasitic and free-living nematodes associated with honebush (*Cyclopia* spp.) and rooibos *(Aspalathus linaeris)* monocultures in the Western Cape province of South Africa.

Families associated with honeybush	c-p values	Families associated with rooibos	c-p values
Alaimidae	4	*Alaimidae*	4
Anguinidae	2	*Anguinidae*	2
Aphelenchoidae	2	*Aphelenchoidae*	2
Aphelenchidae	2	*Aporcelaimidae*	5
Aporcelaimidae	5	*Cephalobidae*	2
Cephalobidae	2	*Diplogasteridae*	1
Criconematidae	(3)	*Discolaimidae*	5
Diplogasteridae	1	*Criconematidae*	(3)
Discolaimidae	5	*Dolichodoridae*	(3)
Dorylaimidae	4	*Dorylaimidae*	4
Elaphonematidae	3	*Elaphonematidae*	3
Heteroderidae	(3)	*Hoplolaiminde*	(3)
Hoplolaiminde	(3)	*Leptonchidae*	4
Leptonchidae	4	*Longidoridae*	5
Longidoridae	5	*Microlaimidae*	2
Microlaimidae	2	*Monhysteridae*	2
Monhysteridae	2	*Nordiidae*	4
Mononchidae	4	*Panagrolaimidae*	1
Nygolaimidae	5	*Pratylenchidae*	(3)
Panagrolaimidae	1	*Plectidae*	2
Plectidae	2	*Rhabditidae*	1
Pratylenchidae	(3)	*Telotylenchidae*	(3)
Rhabditidae	1	*Tripylidae*	3
Telotylenchidae	3	*Tylenchidae*	2
Tylenchidae	2		
Trichodoridae	(4)		

Table classification according to Bongers and Bongers (1998); Bongers (1999).
Values in the bracket indicate plant feeding taxa.
2 $^{(1)}$ = Plant feeding Anguinidae.

The primer sets and cycling condition used, was as described by Adam et al. (2007). The primer set combination Fjav/Rjav and JMV1/JMV2/JMV3 were used for *M. javanica and M. hapla* respectively. The *M. javanica* specific SCAR primers comprised of a forward primer GGTGCGCGATTGAACTGAGC and a reverse primer CAGGCCCTT-CAGTGGAACTATAC (Zijlstra et al., 2000) while *M. hapla* IGS-SCAR primers comprised of CGATGGCGTGCTTTCAAC, TTTCCCCTTAT-GATGTTTACCC and AAAAATCCCCTCGAAAAATCCACC (Wishart et al., 2002). The cycling condition 94 °C for 2 min, followed by 45 cycles of 94 °C for 30 s min, 64 °C for 30 s and 72 °C for 1 min, and one final cycle of 72 °C for 7 min.

PCR to confirm the identity of other important nematode species was done by amplification of the D2D3 expansion segment of the 28S gene of the ribosomal DNA and also a fragment of the cytochrome oxidase gene subunit 1 (*cox 1*) of the mitochondrial DNA. PCR amplification of the D2-D3 expansion segments was carried out with the primer set D2A (5′-ACAAGTACCGTGAGGGAAAGTTG-3′) and D3B (5′- TCGGAAGGAAC-CAGCTACTA-3′), while the forward primer, COIF (5′-GATTTTTTG GKCATCCWGARG-3′) and the reverse primer, XIPHR2 (5′-GTACA-TAATGAAAAT GTG CCAC-3′) were used for the amplification of the cytochrome oxidase gene subunit 1 (*cox1*) of the mitochondrial gene (Daramola et al., 2019). Sequencing of the purified DNA was performed in both directions with the Big Dye Terminator V1.3 sequencing kit, at the DNA Sequencing Unit (Central Analytical Facilities, Stellenbosch University).

2.4. Sequence and phylogenetic analysis

The software for biological sequence alignment editor, Bioedit 7.2 (Hall, 1999) was used for manual sequence assembly and editing. Newly obtained sequences were deposited in GenBank for *Criconema mutabile* Taylor (MK170079), *Scutellonema* sp. (MT371430), *P. bolivianus* (MK170079), *Neodolichorhynchus estherae* Kleynhans (MT371430), and *Xiphinema oxycaudatum* Lamberti & Bleve-Zacheo (MT371430), respectively. The derived DNA sequences were compared for similarity with other sequences obtained on the NCBI using BLASTN (Altschul et al., 1997). Alignment of the sequences was done with Multiple Alignment Fast Fourier Transform (MAFFT). Phylogenetic analyses were conducted with MEGA X version 10.0.5 (Kumar et al., 2018) and the confidence intervals measured using bootstrap (Felsenstein, 1985) with 1000 replicates.

3. Results

3.1. Occurrence and population densities of nematodes

Plant-parasitic nematodes found in association with honeybush monocultures in the Western Cape include; the dagger nematodes, *X. oxycaudatum*, ring nematodes, *C. mutabile*, root-knot nematodes (*Meloidogyne hapla* Chitwood and *Meloidogyne javanica Treub, Chitwood*), spiral nematodes, *Helicotylenchus* spp., *Scutellomema* spp. and the stubby root nematode, *Trichodorus* spp. *Meloidogyne* spp., *Scutellonema* spp. and *X. oxycaudatum* and were recorded in high numbers with mean nematode population densities (MNPD) of 1200, 240, and 220 nematodes per 250ml of soil respectively (Figure 2a). The most frequently encountered plant-parasitic nematodes in the honeybush fields include *Xiphinema, Scutellonema,* and *Meloidogyne* species. They occur at frequencies 73%, 73%, and 55% of samples respectively.

High numbers of the lesion nematode, *Pratylenchus* spp. was recorded from the rooibos monocultures with a mean population density of about

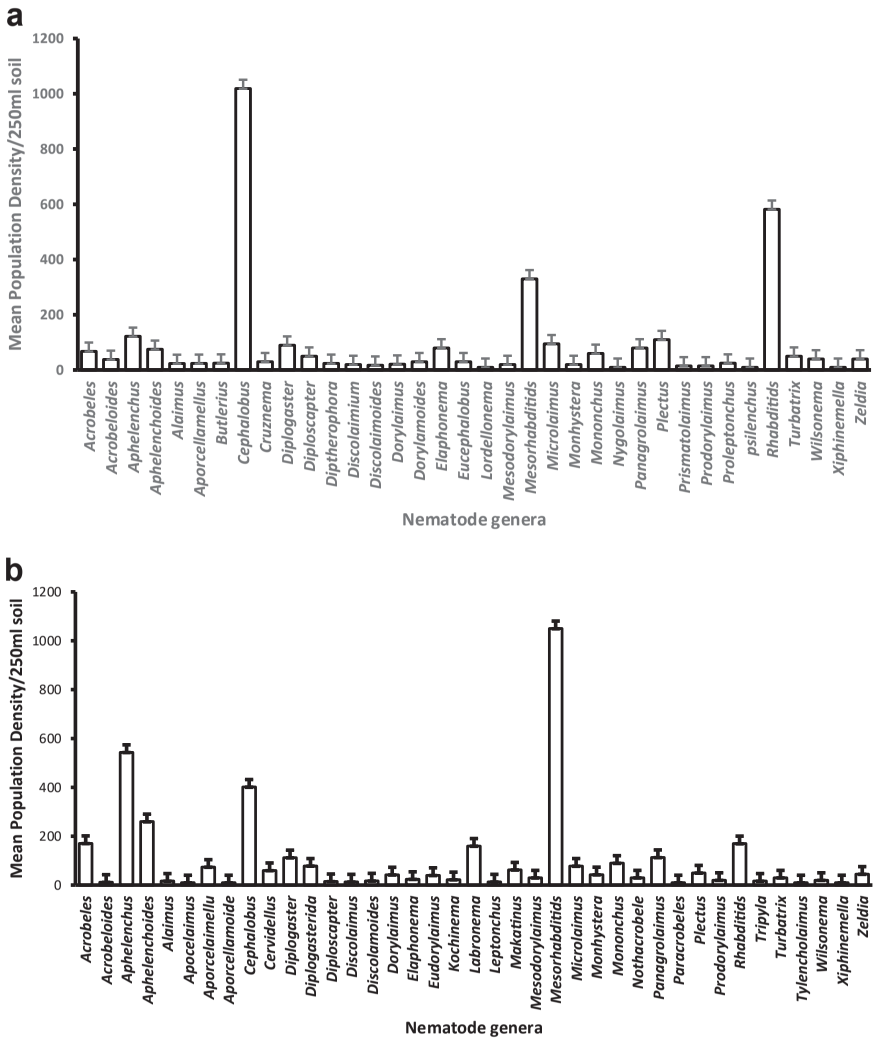

Figure 3. (a) Free-living nematodes associated with honeybush monocultures in the Western Cape Province of South Africa. (a) Free-living nematodes associated with rooibos monocultures in the Western Cape Province of South Africa.

Table 2. Maturity and diversity indices for honeybush and rooibos nematode assemblages.

Indices	Honeybush	Rooibos
MI	1.796	2.01
PPI	3.26	2.76
PPI/MI	1.82	1.37
MI2-5	2.34	2.69
∑MI	2.41	2.21
∑MI2-5	2.84	2.71
Species richness (Hill's N_0 index)	48	51
Shannon's diversity index (H′)	2.87	3.15
Hill's N_1 index	17.64	23.34
Simpson's diversity (D)	0.10	0.07
Hill's N_2 index	10	13.7
Pielou evenness (J′)	0.74	0.80

MI = Maturity index.
PPI = Plant-parasitic index.
MI2-5 = MI excluding c-p1 enrichment opportunists.
∑MI = MI for all nematodes including plant feeder.
∑MI2-5 = MI for all nematodes in the c-p2-5 range (Ferris and Bongers 2009).

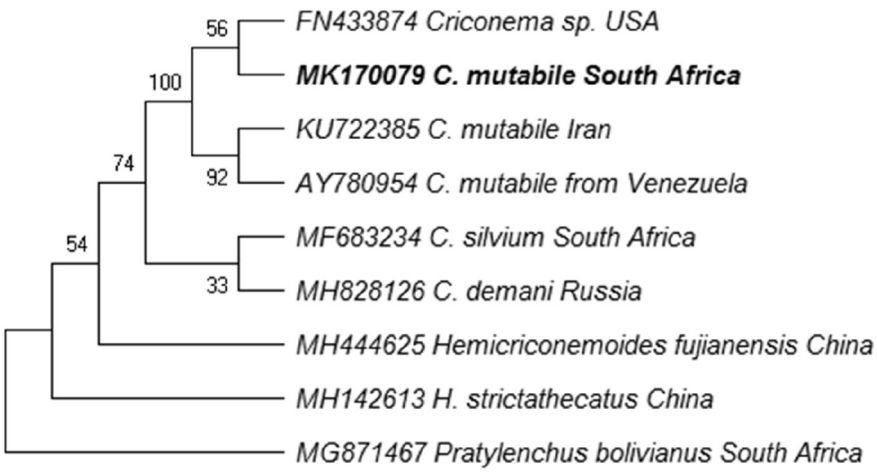

Figure 4. Phylogenetic relationship within closely related *Criconema* species, based on analysis of the D2D3 regions with maximum parsimony (MP) using *Pratylenchus bolivianus* as the outgroup. Newly obtained sequence is indicated in bold print.

700 nematodes per 250 ml of soil in some fields. Also occurring in low population were; *Longidorus* sp., *Hemicycliophora* sp. and *Scutellonema* sp. (Figure 2b). The most frequently encountered nematodes include; *Tylenchus* sp., *Aphelenchus* sp. and *P. bolivianus* occurring at 100%, 92%, and 87% respectively in all the sampled sites.

Twenty-six nematode families were identified from the honeybush fields while twenty-four were identified from the rooibos fields (Table 1). The families were classified based on their c-p values, which ranged from 1-5. The nematodes in each family were assigned c-p values according to their colonizer-persister series (Ferris and Bongers, 2009). Free-living nematodes were abundant and in large numbers on both honeybush and rooibos monocultures. High numbers of bacterial and fungal feeders; *Cephalobus*, *Rhabditids*, and *Mesorhabditids* were recorded from the honeybush fields, and the most frequently encountered families of the free-living nematodes were the *Cephalobidae* and *Rhabditidae* (Figure 3a). They were found in all the samples collected. On the rooibos monocultures, high numbers of *Mesorhabditids, Cephalobus, and Acrobeles* were also recorded on the sampled fields while the most frequently encountered family were Cephalobidae (*Acrobeles* and *Cephalobus*), Monhysteridae, and *Panagrolaimidae*, occurring at 100%, 85%, and 69% respectively (Figure 3b). High numbers of the plant-parasitic nematodes were also recorded with the most frequently encountered families including Tylenchidae and Pratylenchidae and Hoploilamidae. Photomicrographs of representative plant-parasitic and free-living nematodes identified from honeybush and rooibos

3.2. Measures of diversity, richness, and maturity indices

The diversity, richness, and evenness of the nematode species associated with honeybush and rooibos as determined by the various diversity indices are given in Table 2. Shannon's diversity index (H′) indicated values of 2.87 and 3.15 for honeybush and rooibos samples respectively while Simpson's (D) index indicated low values of 0.1 and 0.07 on the tea plants. Hill's N_0 index, indicating species richness was 48 and 51 for honeybush and rooibos respectively and species evenness in both tea plants are close to 1 (0.74 and 0.8) an indication of a very even distribution of abundance amongst species.

Values of the maturity indices (MI), plant-parasitic index (PPI), and the PPI/MI ratio for the nematodes associated with honeybush and rooibos,

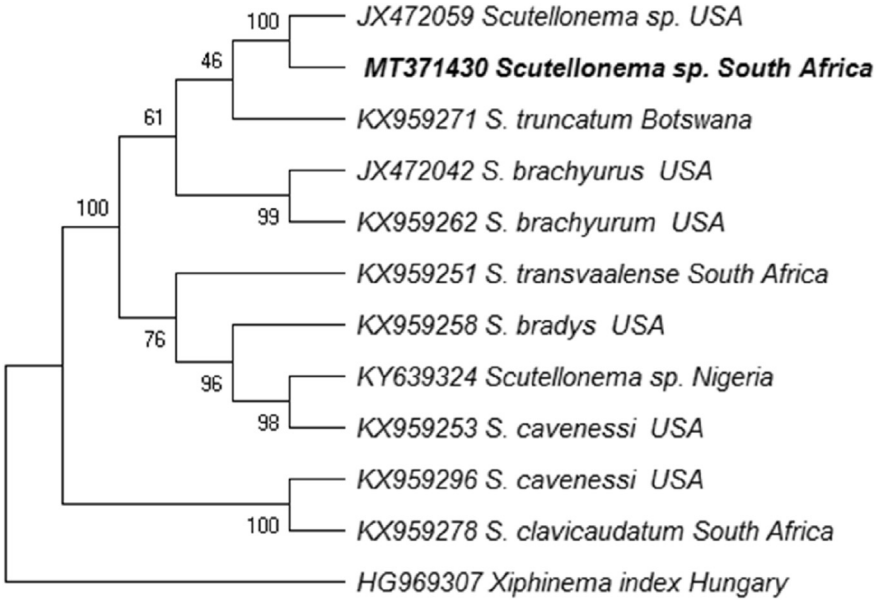

Figure 5. Phylogenetic relationship within closely related *Scutellonema* species, based on analysis of the D2D3 regions with maximum parsimony (MP) using *Xiphinema index* as the outgroup. Newly obtained sequences are indicated in bold print.

are indicated in Table 2. The MI values for honeybush and rooibos nematodes are 1.8 and 2.0 respectively, indicating a disturbed and/or enriched environment. PPI values are 3.26 and 2.76 and low PPI/MI ratios (1.8 and 1.4) were also recorded, indicating poor nutrient conditions.

3.3. Molecular identification

Molecular identification of the root-knot nematodes of honeybush with SCAR primers showed that two species of the root-knot nematode; *M. hapla* and *M. javanica* occur on honeybush. The PCR products obtained from the DNA amplification of the populations of the two *Meloidogyne* species produced PCR products of 670 and 720 base pairs (bp) for *M. hapla* and *M. javanica* respectively. From the analysis of the DNA sequences derived from other plant-parasitic nematodes of the tea plants, *C. mutabile* (MK170079) with 747 bp gave a similarity of 99.97% to *C. mutabile* (AY780954) from Venezuela; *Scutellonema* sp. (MT371430) with 746 bp showed a 100% similarity to those described from the USA (JX472059), and *P. bolivianus* (MG871467) with 764 base pairs corresponds at 99.87% similarity to other *P. bolivianus* sequences (KU198955; KU198956) from the GenBank. Other submitted sequences include those of *N. estherae* and *X. oxycaudatum* with accession numbers M288016 and MK211480 respectively. However, there were no available sequences for their comparison on the GenBank. The result of the phylogenetic analyses and evolutionary history of the plant-parasitic nematodes as inferred using the maximum parsimony method is given in Figures 4, 5, and 6.

3.4. Free-living nematodes

Twenty-two families of free-living nematodes were identified from the honeybush monocultures and nineteen from the rooibos monocultures (Table1). Based on their feeding groups, the nematodes were grouped into bacterivores, fungivores, predacious, and omnivores. The nematodes in each family were assigned cp values according to their colonizer-persister series (Ferris and Bongers, 2009). Photomicrographs of representative plant-parasitic and free-living nematodes identified from honeybush and rooibos monocultures are shown in Figures 7, 8, and 9.

4. Discussion

Plant-parasitic nematodes are important pests causing reduced crop yield and economic losses on agricultural crops worldwide. In the current study, *M. hapla, M. javanica, C. mutabile, Scutellonema sp.* and *X. oxycaudatum* are identified as important pests of honeybush, while the root-lesion nematode, *P. bolivianus* is abundant and pathogenic on rooibos monocultures. These nematodes have been reported as major constraints, limiting the productivity of agricultural products worldwide (Cotten et al., 1991; Jones et al. 2013). According to Coyne et al. (2018) root-knot nematodes (*Meloidogyne* spp.) and the lesion nematodes (*Pratylenchus* spp.) are the two most important groups of nematodes and can infect, feed on, and reproduce on an astonishing range of crops and plant species. They considered *Meloidogyne* species as the greatest biotic threat to crop production in sub-Saharan Africa.

Damage due to root-knot nematodes have been reported on tea, *Camellia sinensis* (L) Kuntze (Kamunya et al., 2008; Orisajo, 2013) *M. hapla*, and *M. javanica* were pathogenic on honeybush tea in the current investigation. Severe galling on the plant roots were evident and could cause damage to the root system, disrupting the ability of the root cells to absorb water and hinder efficient uptake of nutrients for plant growth and development. In some cases, severely aberrated root systems could lead to the death of plants.

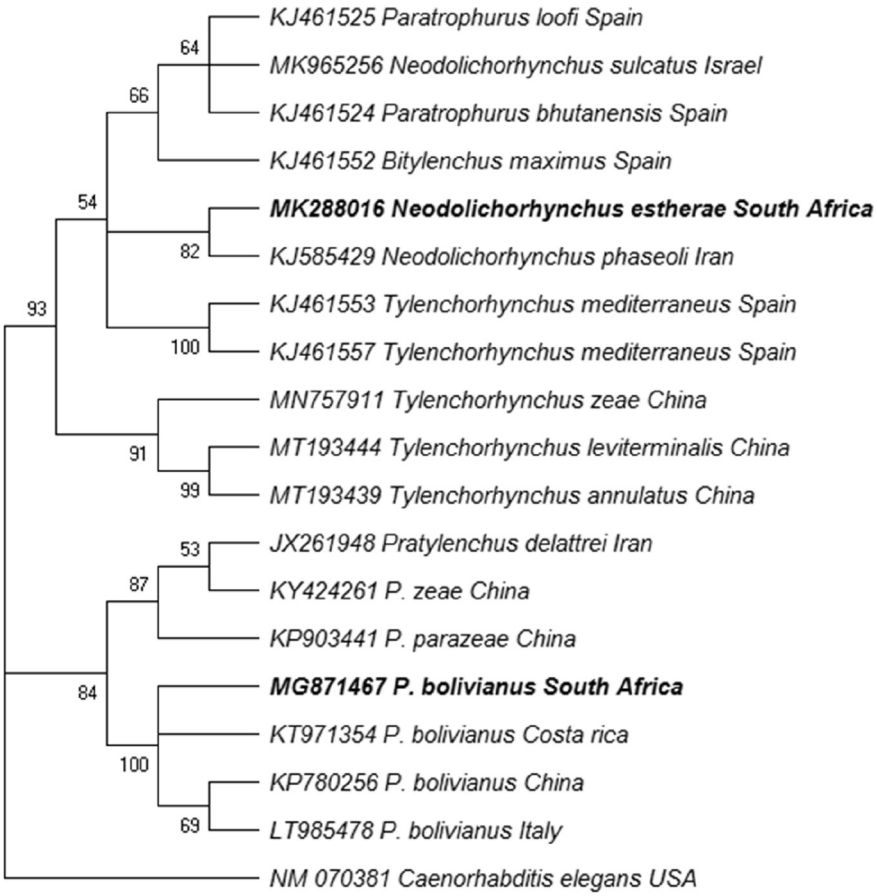

Figure 6. Phylogenetic relationship within closely related species of *Pratylenchus* and *Neodolychorynchus*, based on analysis of the D2D3 regions with maximum parsimony (MP) using *Caenorhabditis elegans* as the outgroup. Newly obtained sequences are indicated in bold print.

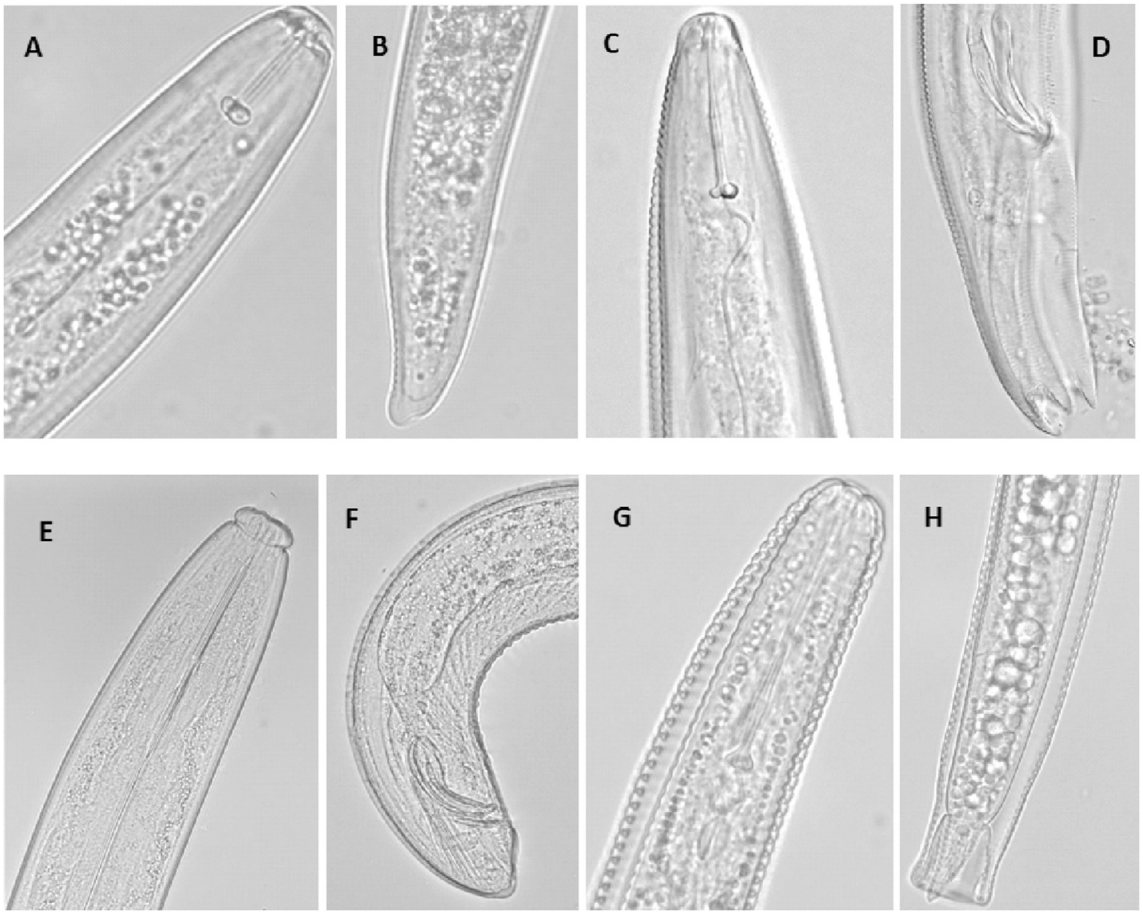

Figure 7. Photomicrographs of some plant-parasitic nematodes associated with rooibos monocultures (A–B). *Pratylenchus bolivianus* (C–D). *Neodolichorhynchus estherae,* (E–F). *Dolichodorus* sp. and (G–H). *Hemicycliophora* sp.

The lesion nematode, *P. bolivianus* (Corbet, 1983)) is another important plant-parasitic nematode that was recorded in high numbers from the rooibos fields investigated in this study. *P. bolivianus* is a highly pathogenic nematode that was originally described from the soil around the roots of oats and potatoes in the Bolivian Andes (Corbet, 1983). This nematode was also reported in the Netherlands to be causing serious damage to tomatoes and carnation (Cotten et al., 1991). The presence of this nematode species in high numbers on rooibos monocultures is of great concern.

Other plant-parasitic nematodes reported in the honeybush fields such as *Criconema mutabile, X. oxycaudatum*, *Trichodorus* sp., *Scutellonema* sp., *Hoplaimus* sp, and *Helicotylenchus* sp. are important nematodes that could lead to a reduction in crop productivity, reduce plant vigour due to their feeding activities and could also form disease complexes with other pathogens. Some species of *Xiphinema* have also been implicated as vectors, transmitting plant viruses (Brown and Halbrendt, 1992). In India, Gnanapragasam and Mohotti (2005) reported some nematode species that are either known or suspected to be pathogenic on tea plants. They include; *Pratylenchus* spp., *Radopholus similis* (Cobb) Thorne, *Meloidogyne* spp, *Hemicriconemoides kanayaensis* Nakasono & Ichinohe, *Rotylenchulus reniformis* Linford & Oliveira, *Helicotylenchus* spp. *Paratylenchus curvitatus* van der Linde, *Hoplolaimus sp. Rotylenchus sp.* and *Xiphinema* sp. The result of this survey also agrees with the report of Orisajo (2013) who indicated *Meloidogyne spp*, *Xiphinema spp*, and *Helicotylenchus coffeae* Zimmermann as dominant nematode species associated with tea plants in Nigeria.

In terms of nematode abundance and diversity, more nematodes were recorded on the honeybush orchards in comparison to the rooibos monocultures. However, the rooibos samples were more diverse, richer in species, and are also more evenly distributed as indicated by Shannon's diversity index (H′), Hill's N_0 index, and Pielou's evenness J′ index. These diversity indices provide an assessment of the heterogeneity of the soil ecosystem. In the current investigation, the most abundant trophic groups under the tea plants are the bacterivorous nematodes comprising of the families cephalobidae and rhabditidae. This is followed by herbivores and fungivores while lower numbers of omnivores and predators were observed. Similar reports have been documented on many agroecosystems (Tsiafouli et al., 2006; Kapp et al., 2013; Girgan et al., 2020).

The maturity indices of free-living nematodes have been described as useful indicators of soil environmental health. They have been extensively used as bio-indicators of soil diversity and functioning (Neher, 2001; Mulder et al., 2005). In the current study, the MI values for both honeybush and rooibos monocultures are low, thereby indicating a disturbed or enriched condition. Similar reports have been recorded on some fynbos monocultures and grasslands in South Africa (Kapp et al., 2013); Girgan et al., 2020). From the study, it was observed that the Maturity indices for the free-living nematodes were low for both tea plants, although it was slightly higher on rooibos than the honeybush orchards. However, the reverse was observed for the plant-parasitic index (PPI) which was higher than the MI for both honeybush and rooibos orchards. Higher MI values indicate a less disturbed soil condition, which is typified by the presence of more persisters such as omnivores and predatory nematodes as observed in the rooibos monocultures. Higher MI values in the system also indicate a higher diversity of high c–p value nematodes and a more stable ecosystem (Grewal et al., 2011). From the study, the Plant-parasitic index (PPI) was lower on rooibos. This result is expected since the PPI is lower under enriched agricultural conditions (Ferris and Bongers, 2009) and

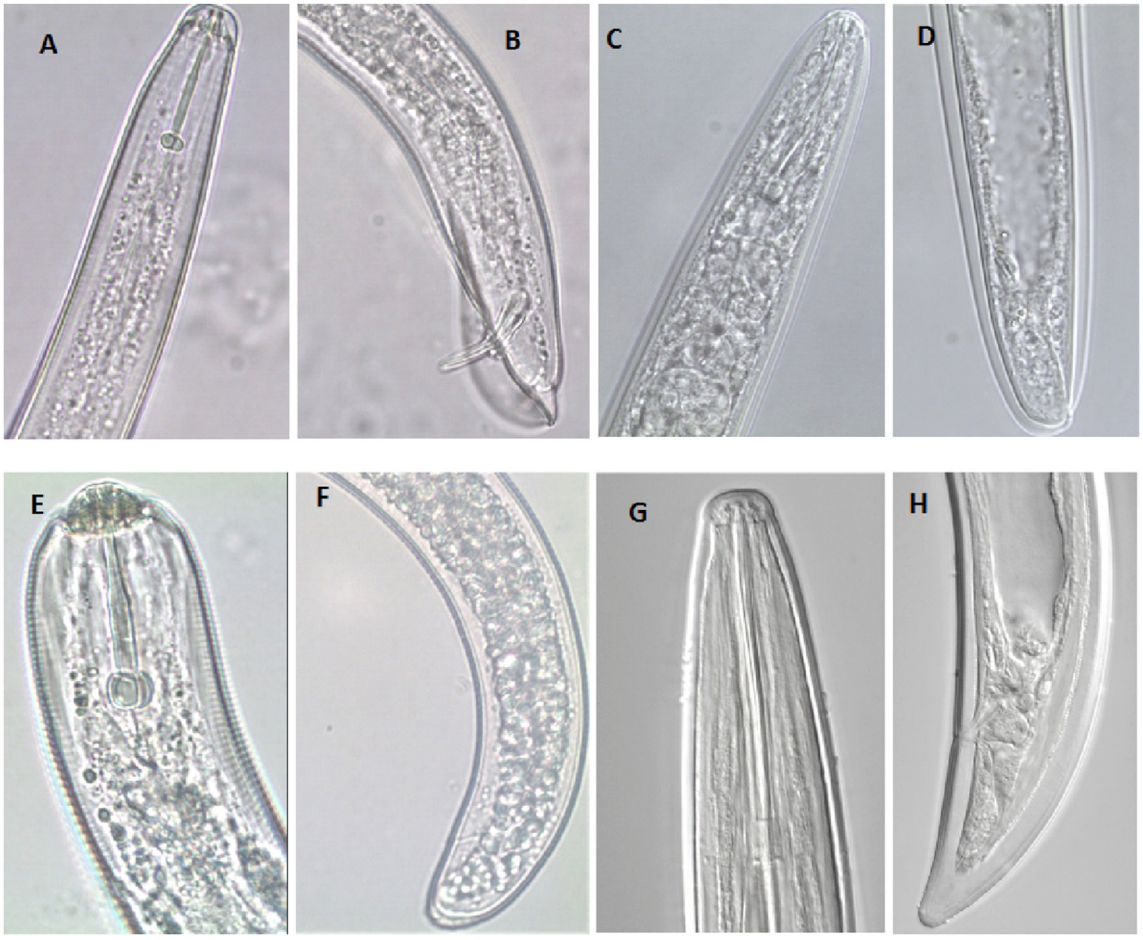

Figure 8. Photomicrographs of the head and tail region of some plant-parasitic nematodes of honeybush in Western Cape province of South Africa. (A–B) *Scutellonema* sp., (C–D). *Trichodorus* sp., (E–F). *Hoplolaimus* sp. and (G–H) *Xiphinema oxycaudatum*.

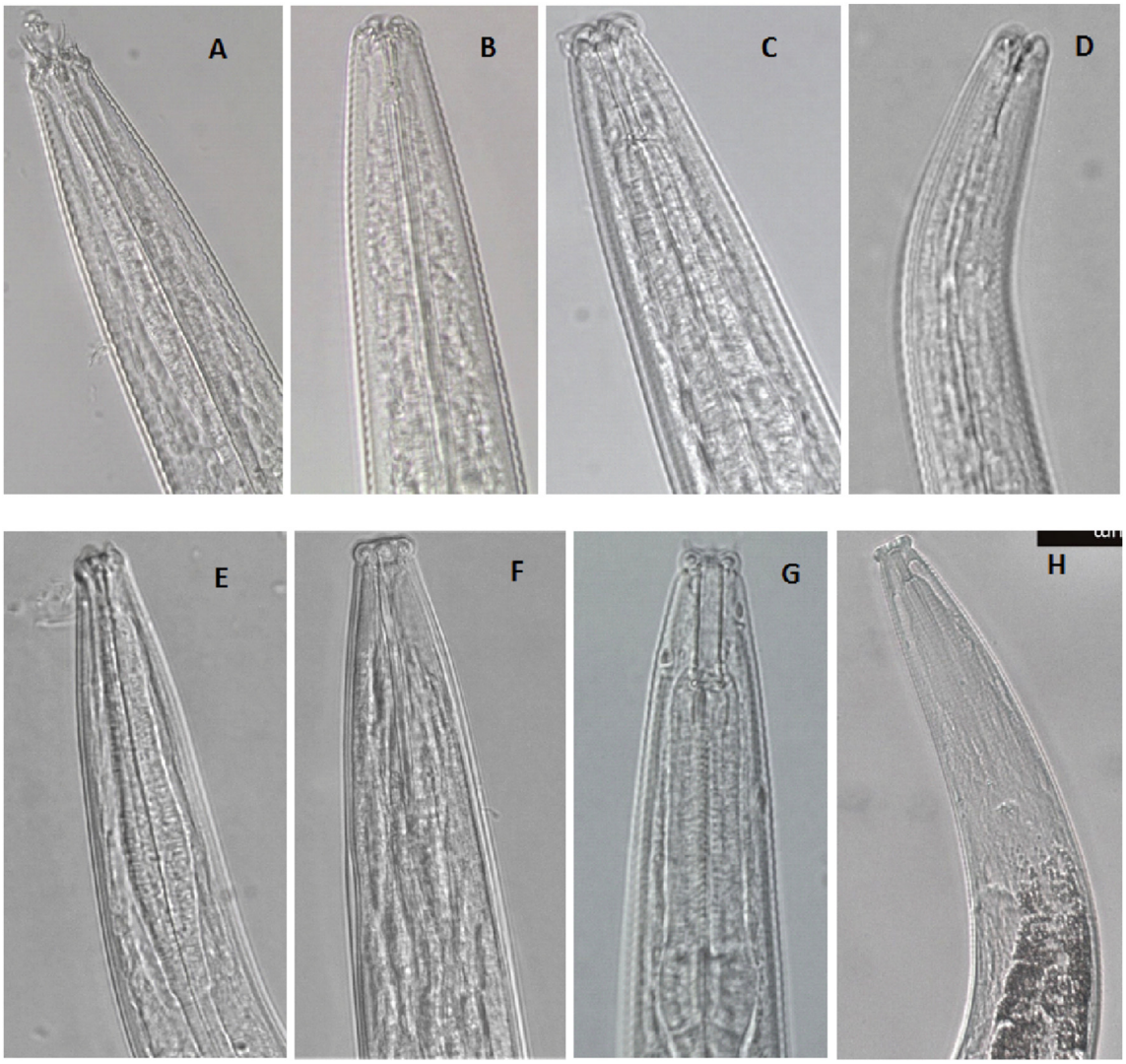

Figure 9. Photomicrographs of the head region of some free-living nematodes from honeybush and rooibos monocultures. (A) *Acrobeles* (B) *Pseudacrobeles* (C) *Cruznema* (D) *Cephalobus* (E) *Acrobeloides* (F) *Aporcelaimus* (G) *Mesorhabditid* (H) *Rhabditid*.

more plant-parasitic nematodes were recorded on the honeybush orchards. Daramola et al. (2020) in a previous study has reported the susceptibility of honeybush to plant-parasitic nematodes including the root-knot nematodes, Meloidogyne species. The PPI/MI ratio is low on both fields but slightly higher on the honeybush fields, also indicating a more enriched and disturbed soil condition of the honeybush orchards.

According to Bongers and Ferris (1999), nematodes occupy key positions in soil food webs and can influence vegetation succession through their feeding activities. An assessment of the families of the free-living nematodes from the honeybush and rooibos monocultures in the present study indicated high numbers of the bacterial and fungal feeders (*Rhabditidae* and *Cephalobidae*) with a lower population of the predators and omnivores, being recorded. This might suggest a low C: N ratio and a disturbed food web condition, which is indicative of disturbance in the soil nematode community. Yeates and Hughes (1990), postulated that soil disturbance could result in a reduction in the number of nematode genera in the soil, therefore climate changes, resulting from intensive agricultural activities could lead to reduced diversity, creating an imbalance in the food web and the nematode assemblages within the soil ecosystem.

Further comparative studies and analyses of nematode community structures under different agronomic practices, if conducted over a long duration of time could provide useful insights into the changes in the nematode assemblages of honeybush and rooibos plantations in South Africa. This might be crucial in order to take relevant conservation and management decisions in preserving the endangered indigenous South African tea plants.

Declarations

Author contribution statement

Fisayo Y Daramola: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Francis B. Lewu: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Antoinette P. Malan: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This work was supported by the National Research Foundation (grant no: 99679).

Data availability statement

Data associated with this study has been deposited at GenBank Accession numbers MG871467, MK170079, MT371430, M288016 and MK211480.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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