Use of Essential Oils as Phytonematicides on the Management of Plant parasitic nematodes in South Africa

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AGRICULTURAL RESEARCH COUNCIL

Introduction

DoA core mandate:

- Sustainable agricultural development, land reform, and rural socio-economic growth.
- Increasing agricultural productivity and assisting rural communities.
- To reduce plant-parasitic nematodes (PPN) and increase productivity of vegetables.
- Develop pest and disease products to empower emerging-smallholder farmers, extension services and agricultural advisors

Essential Oils (EOs): wide range of properties and biological activities

- **Rosemary** (*Rosmarinus officinalis*)
- **Rose geranium** (*Pelargonium graveolens*)
- **Thyme** (*Thymus vulgaris*)
- **Fever tea/Lemon bush** (*Lippia javanica)*
- **Ginger** (*Zingiber officinale*)
- **Marjoram** (*Origanum majorana)*
- **Lavender** (*Lavandula spica*)
- **Eucalyptus** (*Eucalyptus obliqua)*
- **Cinnamon** (*Cinnamomum verum*)
- **Oregano** (Origanum vulgare)

Bioassays trials:

- Department of Paraclinical Sciences, University of Pretoria, Onderstepoort Campus, and ARC-Tropical & Subtropical Crops, Mbombela Campus
- Plant Parasitic Nematodes: Root-knot nematodes (*Meloidogyne incognita, M. javanica* and *M. enterolobii*) species

Preparation of stock solutions:

Stock solutions of each EOs were prepared at 8 000 ppm.

- Serial dilutions included concentrations of 4 000, 2 000, 1 000, 500, 250, 125, 63, 32, 16, 8, 4, 2,1, 0.5 ppm.

Effect on J2 mortality and egg-hatching bioassays:

- A 100 µl suspension of distilled water containing 100 ± 20 freshly-collected J2 or eggs was added to each 96-well plate.
- The treatments were assigned in triplicate in a completely randomized design.
- Three controls were included: a solution of Tween-80 (0.1%) , 10 % MeOH/Tween-80 and 1 mg ml^{-1} salicylic acid dissolved in 10 % MeOH/Tween-80.
- The assay was repeated once in triplicates.
- Evaluations for **mortality** at 24-, 48- and 72-h; for **egg-hatching** at 7-, 14- and 21-days.

Table 1. Efficacy of *Lippia javanica* and Cinnamon oil extracts concentrations in ppm and three control treatments after 24-, 48- and 72- hours (h) exposure times on viability of second-stage juveniles (J2) *of Meloidogyne incognita* under in vitro condition.

		***Meloidogyne incognita*: J2's mortality (%)**					
		***Lippia javanica* oil**			**Cinnamon oil**		
Treatment	**Extract**	**24 h**	**48 h**	**72 h**	**24 h**	**48 h**	**72 h**
A	Salicylic acid	114.0 a	114.0 a	113.3 a	107.0 a	107.0 a	107.0 a
B	10% MeOH	2.5 f	3.5 h	4.8 e	6.7 e	7.7 f	10.2 e
C	Tween-80	3.0 f	3.3 h	5.5 e	5.0 e	6.8 f	9.2 e
G	1 000 ppm	57.7 c	111.7 a	111.7 a	106.5 a	106.5 ab	106.5 a
H	500 ppm	17.2 e	77.5 b	114.0 a	104.8 a	104.8 ab	104.8 ab
I	250 ppm	4.5 f	24.7 c	41.3 b	107.0 a	107.0 a	107.0 a
J	125 ppm	2.8 f	11.8 fg	24.8 c	83..8 b	97.3 c	105.0 ab
K	63 ppm	23.8 d	20.7 cd	19.8 c	104.0 a	104.0 ab	104.0 ab
L	32 ppm	15.7 e	18.2 de	14.7 d	100.0 a	102.2 b	102.2 b
M	16 ppm	6.0 f	15.5 ef	14.0 d	65.3 c	52.5 d	48.8 c
N	8 ppm	5.5 f	11.7 fg	12.0 d	22.3 d	21.5 e	16.5 d
O	4 ppm	2.0 f	9.5 g	10.0 de	5.0 e	8.2 f	10.3 e
	$LSD_{0.05}$	7.8173	5.1278	5.6335	7.6250	4.4905	4.1249
	P-value	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	F-value	649.49	708.58	621.81	270.00	787.43	942.92

Percentages in the same column followed by a different letter are significantly (P ≤ 0.05) different according to Fisher's protected least significant difference (LSD) test.

Table 2. Efficacy of *Lippia javanica* and Cinnamon oil extracts concentrations in ppm and three control treatments after 7-, 14- and 21- days exposure on hatching of second-stage juveniles (J2) of *Meloidogyne incognita* under in vitro condition.

		***Meloidogyne incognita*: Egg hatching (%)**					
		***Lippia javanica* oil**			**Cinnamon oil**		
Treatment	**Extract**	**7-days**	**14-days**	**21-days**	**7-days**	**14-days**	**21-days**
A	Salicylic acid	10.3 e	10.3 e	10.5 e	17.5 a	20.3 cd	21.2 c
B	10% MeOH	16.0 e	56.8 c	56.8 c	16.5 a	20.5 cd	66.3 a
C	Tween-80	15.2 e	56.2 c	56.3 c	16.2 a	24.2 c	62.7 ab
G	1 000 ppm	11.3 e	11.3 de	11.7 de	9.3 b	11.8 e	11.8 d
H	500 ppm	17.5 e	17.5 d	18.2 d	13.3 ab	19.5 cde	26.7 c
I	250 ppm	42.3 cd	56.5 c	57.2 c	11.5 ab	16.0 de	25.8 c
J	125 ppm	38.0 d	58.8 c	60.8 c	11.3 ab	14.3 de	22.3 c
K	63 ppm	47.8 bcd	69.3 b	70.3 b	13.0 ab	16.8 cde	23.5 c
L	32 ppm	45.2 bcd	69.7 b	70.2 b	13.7 ab	16.7 cde	21.3 c
M	16 ppm	50.3 b	71.5 ab	71.7 ab	12.5 ab	19.5 cde	23.0 c
N	8 ppm	62.2 a	76.0 ab	76.2 ab	16.5 a	32.8 b	55.0 b
O	4 ppm	61.0 a	77.0 a	77.3 a	14.8 ab	41.2 a	57.8 ab
	$LSD_{0.05}$	7.5313	6.7405	6.7775	6.3928	7.7509	8.9001
	P-value	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	F-value	55.12	131.80	131.49	1.22	9.10	37.82

Percentages in the same column followed by a different letter are significantly ($P \leq 0.05$) different according to Fisher's protected least significant difference (LSD) test.

Oil extract	*M. incognita* Concentration (ppm) and Motility (%) after 72-h											
	8 000	4 000	2 000	1 000	500	250	125	63	32	16	8	4
Cinnamon	107	107	107	107	105	107	105	104	102	102	49	-
Oregano	102	102	102	105	104	84	75	26	16	-	-	-
Lippia javanica	114	112	114	112	114	41	-	-	-	-	-	-
Marjoram	109	107	56	25	-	-	-	-	-	-	-	-
Eucalyptus	111	106	41	-	-	-	-	-	-	-	-	-
Thyme	106	90	49	-	-	-	-	-	-	-	-	-
Ginger	92	87	49	-	-	-	-	-	-	-	-	-
Lavender	85	62	45	-	-	-	-	-	-	-	-	-
Rosemary	-	-	-	-	-	-	-	-	-	-	-	-
Rose geranium	-	-	-	-		-	-	-	-	-	-	-

Oil extract	*M. Incognita* Concentration (ppm) and hatching (%) after 21-days											
	8 000	4 000	2 000	1 000	500	250	125	63	32	16	8	4
Cinnamon	13	13	13	13	14	16	20	7	11	32	-	-
Lippia javanica	15	14	12	12	18	-	-	-	-	-	-	-
Oregano	13	14	10	12	15	13	-	-	-	-	-	-
Thyme	12	10	11	18	36	-	-	-	-	-	-	-
Marjoram	16	16	31	27	31	-	-	-	-	-	-	-
Rose geranium	13	15	26	43	-	-	-	-	-	-	-	-
Lavender	18	27	37	41	-	-	-	-	-	-	-	-
Eucalyptus	33	29	45	42	-	-	-	-	-	-	-	-
Ginger	42	45	45	-	-	-	-	-	-	-	-	-
Rosemary	-	-	-	-	-	-	-	-	-	-	-	-

Oil extract	Discussion and Conclusion: Mortality active and potent concentration level		
	Meloidogyne incognita	***M. enterolobii***	***M. javanica***
Cinnamon	8 000-16 ppm	8 000-32 ppm	8 000-32 ppm
Lippia javanica	8 000-500 ppm	1 000-125 ppm	8 000- 125 ppm
Oregano	8 000-125 ppm	1 000-125 ppm	8 000- 125 ppm
Thyme	8 000-4 000 ppm	8 000-1 000 ppm	8 000-1 000 ppm
Lavender	8 000-4 000 ppm	8 000-1 000 ppm	8 000-500 ppm
Eucalyptus	8 000-4 000 ppm	1 000-125 ppm	8 000-1 000 ppm
Marjoram	8 000- 2 000 ppm	1 000-125 ppm	8 000-1 000 ppm
Ginger	8 000- 2 000 ppm	8 000-500 ppm	-
Rose geranium	-	8 000-4 000 ppm	8 000 ppm
Rosemary	-	8 000-1 000 ppm	8 000 ppm

Oil extract	Discussion and conclusion: Egg hatching active and potent concentration level		
	Meloidogyne incognita	***M. enterolobii***	***M. javanica***
Cinnamon	8 000- 250 ppm	-	8 000- 16 ppm
Lippia javanica	8 000- 500 ppm	8 000- 500 ppm	8 000- 1 000 ppm
Oregano	8 000-125 ppm	8 000-125 ppm	8 000- 125 ppm
Thyme	8 000-2 000 ppm	8 000- 500 ppm	8 000- 1 000 ppm
Lavender	-	8 000- 4 000 ppm	-
Eucalyptus	-	8 000- 4 000 ppm	8 000-500 ppm
Marjoram	8 000- 4 000 ppm	8 000- 2 000 ppm	8 000- 4 000 ppm
Ginger	8 000- 4 000 ppm	8 000 ppm	8 000 ppm
Rose geranium	8 000- 4 000 ppm	8 000- 2 000 ppm	-
Rosemary	-	-	-

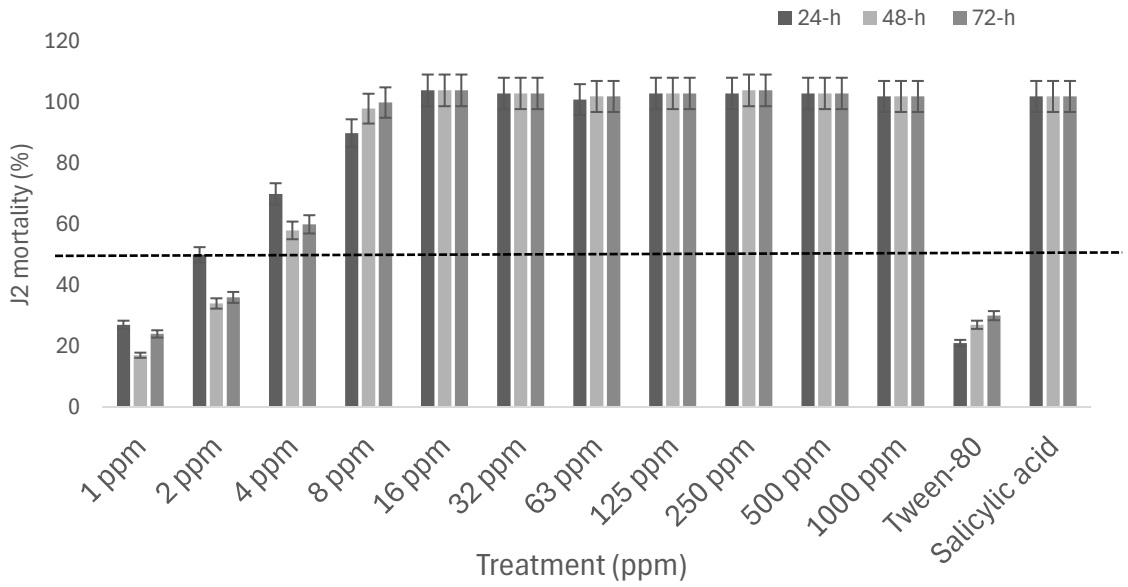

Figure 1: Blended Essential Oil extracts of Cinnamon, Oregano and Fever tea (1:1:1 v/v/v) with carriers on Mortality second-stage juveniles (J2) of *Meloidogyne incognita* at 24-h, 48-h and 72-h exposure times under *in vitro* conditions

List	Common name	Metabolites class against PPN	GCMS analysis: CSIR
1	Rosemary	α-pinene, 1,8-cineole, camphor, flavonoids, phenolics, terpenoids	Monoterpenoids: α-Phellandrene ($C_{10}H_{16}$), Piperitone ($C_{10}H_{16}O$), 4,4-Dimethyl-3-(3-methylbut-3-enylidene)-2 methylenebicyclo[4.1.0] heptane ($C_{15}H_{22}$), ß-Myrcene ($C_{10}H_{16}$), ß-Terpinyl acetate ($C_{12}H_{20}O_2$), (+)-Sabinene ($C_{10}H_{16}$), Phellandral ($C_{10}H_{16}O$), α-Pinene ($C_{10}H_{16}$), Cycloalkanes: Methylcyclopentane (C_6H_{12})
2	Thyme	Monoterpenoids: geraniol, linalool, terpinen-4-ol, α-terpineol, Thymol, Carvacrol	Monoterpenoids: α-Pinene ($C_{10}H_{16}$), Camphene ($C_{10}H_{16}$), α-Terpine ($C_{10}H_{16}$), endo-Borneol ($C_{10}H_{18}O$), Cresols: Carvacrol methyl ether ($C_{11}H_{16}O$), Methyl thymol ether ($C_{11}H_{16}O$), Sesquiterpenoids: Caryophyllene ($C_{15}H_{24}$), Clovene ($C_{15}H_{24}$), Oxanes: Eucalyptol ($C_{10}H_{18}O$), Cycloalkanes: Methylcyclopentane (C_6H_{12})
3	Marjoram	flavonoids, phenolics, terpenoids	Monoterpenoids: (1S)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene ($C_{10}H_{16}$), o-Cymene ($C_{10}H_{14}$), α-Thujene ($C_{10}H_{16}$), 3-Carene ($C_{10}H_{16}$), ß-Myrcene ($C_{10}H_{16}$), D-Limonene ($C_{10}H_{16}$), Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester ($C_{12}H_{20}O_2$), Camphene ($C_{10}H_{16}$), Anisoles: 1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)- ($C_{11}H_{12}O_3$), Elemicine ($C_{12}H_{16}O_3$)
4	Lavender	Linalool, linalyl acetate	Fatty alcohols: 2,5-Dimethyltetrahydrofuran ($C_6H_{12}O$), Fatty acids esters: 2-Cyclohexen-1-ol, 3-methyl-6-(1-methylethyl)-, acetate ($C_{12}H_{20}O_2$), Monoterpenoids: α-Pinene ($C_{10}H_{16}$),o-Cymene ($C_{10}H_{14}$), (-)-Camphor ($C_{10}H_{16}O$), endo-Borneol ($C_{10}H_{18}O$), α-Terpineol ($C_{10}H_{18}O$), Linalyl iso-valerate ($C_{15}H_{26}O_2$), Lavandulyl acetate ($C_{12}H_{20}O_2$)
5	Rose geranium	Linalool	Cycloalkanes: Methylcyclopentane (C_6H_{12}), Pyrrolopyrazines: 4-Aminobenzyl cyanide ($C_8H_8N_2$), Cinnamaldehydes: (Z)-3-Phenylacrylaldehyde (C_9H_8O), Methoxyphenols: Phenol, 2-methoxy-3-(2-propenyl)- ($C_{10}H_{12}O_2$), Benzoic acid esters: Benzyl Benzoate ($C_{14}H_{12}O_2$), Monoterpenoids: (-)-ß-Pinene ($C_{10}H_{16}$), D-Limonene ($C_{10}H_{16}$), Sesquiterpenoids: Caryophyllene ($C_{15}H_{24}$),Humulene ($C_{15}H_{24}$)
6	Fever tea /Lemon bush	Volatile Organic Compounds (VOCs): benzene acetaldehyde, 2-nonanone, decanal, 2-undecanone, dimethyl disulphide, Terpenes: myrcene, carvone, limonene, linalool; Alkaloids; Flavonoids	Monoterpenoids: α-Pinene ($C_{10}H_{16}$), (+)-Sabinene ($C_{10}H_{16}$), α-Terpineol ($C_{10}H_{18}O$), Sesquiterpenoids: Copaene ($C_{15}H_{24}$), (-)-Cedrene ($C_{15}H_{24}$), Humulene ($C_{15}H_{24}$), γ-Muurolene ($C_{15}H_{24}$), (+)-δ-Cadinene ($C_{15}H_{24}$), (+)-ß-Costol ($C_{15}H_{24}O$)
7	Ginger	10-gingerol, 6-dehydroshogaol, 6-dehydro-10-gingerol	Cycloalkanes: Methylcyclopentane (C_6H_{12}), Oxanes: Eucalyptol ($C_{10}H_{18}O$), Monoterpenoids: Tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl- ($C_{10}H_{16}$), Camphene ($C_{10}H_{16}$), (-)-ß-Pinene ($C_{10}H_{16}$), Isovaleric acid, linalyl ester ($C_{15}H_{26}O_2$), cis-p-Mentha-2,8-dien-1-ol ($C_{10}H_{16}O$), Sesquiterpenoids: Curcumene ($C_{15}H_{22}$), (-)-Zingiberene ($C_{15}H_{24}$), ß-Bisabolene ($C_{15}H_{24}$)
8	Eucalyptus	monoterpene 1,8-cineole, hydrocarbon monoterpene, *p*-cymene	Cycloalkanes: Methylcyclopentane (C_6H_{12}), Branched unsaturated hydrocarbons: γ-Terpinene ($C_{10}H_{16}$), Monoterpenoids: Cyclene ($C_{10}H_{16}$), ß-Myrcene ($C_{10}H_{16}$), α-Terpinene ($C_{10}H_{16}$), *p*-Cymene ($C_{10}H_{14}$), α-Terpineol ($C_{10}H_{18}O$), Piperitone ($C_{10}H_{16}O$), (-)-Bornyl acetate ($C_{12}H_{20}O_2$)
9	Cinnamon	Trans-cinnamaldehyde (CA), Proanthocyanidins (PAC)	Fatty alcohol esters: Citronellyl formate ($C_{11}H_{20}O_2$), Fatty acid esters: 2-Phenylethyl tiglate ($C_{13}H_{16}O_2$), Monoterpenoids: Cyclofenchene ($C_{10}H_{16}$), *p*-Cymene ($C_{10}H_{14}$), Isovaleric acid ($C_{15}H_{26}O_2$), Sesquiterpenoids:Copaene ($C_{15}H_{24}$), Caryophyllene ($C_{15}H_{24}$), Humulene ($C_{15}H_{24}$), γ-Cadinene ($C_{15}H_{24}$)
10	Oregano	Tannins, gallic tannins, saponins, glucosides, flavonoids	???

Non-crop plant species with herbal or medicinal properties on the management of Plant parasitic nematodes in South Africa:

Plant name	Scientific name	Family	Management
Sjambok pod	*Cassia abbreviata* Oliv.	Leguminosae	Phytotoxic
Cucumber cactus	*Cissus cactiformis* Gilg.	Vitaceae	Phytotoxic
L.C. Wheeler, 'Naboom' or Candelabra tree	*Euphorbia ingens* E.Mey.	Euphorbiaceae	Phytotoxic
Morning glory	*Ipomoea kituiensis* Vatke	Convolvulaceae	Phytotoxic
Bead-bean	*Maerua angolensis* DC.	Capparaceae	Potent
Wild Senna	*Senna petersiana* (Bolle) Lock.	Leguminosae	Phytotoxic
Beadman's Tree	*Synadenium cupulare* (Boiss.) Wheeler ex A.C. White, R.A. Dyer and B. Sloane	Euphorbiaceae	Phytotoxic
Toad tree	*Tabernaemontana elegans* Stapf.	Apocynaceae	Potent
Slangkop	*Urginea sanguinea* Shinz.	Hyacinthaceae	Phytotoxic

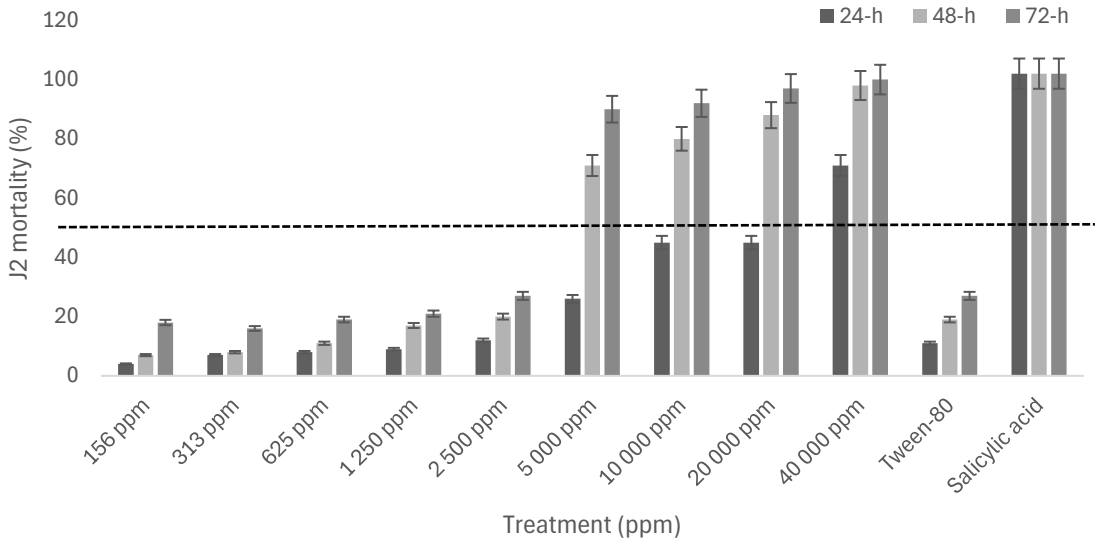

Figure 2: Mortality of second-stage juveniles (J2) *Meloidogyne incognita* after 24-h, 48-, and 72-h exposure times to MeOH/DCM extract of blended *Tabernaemontana elegans* and *Maerua angolensis* (1:1 v/v) under *in vitro* conditions

Mode of Action:

- **Disruption of the Nervous System**: paralysis.
- **Cell Membrane Damage**: causing structural damage and leading to cell lysis.
- **Inhibition of Egg Hatching**: EOs penetrate the gelatinous matrix surrounding nematode eggs and interfere with the hatching process.

Prospects:

- Student (MSc), University of Pretoria.
- Student (PhD), University of Limpopo
- Statistical analysis of the Lethal Concentration (LC_{50}) and Curve-fitting Allelochemical Response Dosage (CARD) computer-based model.
- Assessment of nematode reproduction, vegetative growth and yield of the vegetable plants in the glasshouse, microplot and field conditions.
- Determination of residues of applied natural nematicides in plant tissues and their genotoxicity, phytotoxicity and cytotoxicity to microsomal and animal tissues.
- Field demonstration trials, Workshops and farmers days in Limpopo, Mpumalanga and KwaZulu-Natal Provinces

Collaborators:

- **Prof. Stephen Amoo:** ARC-Vegetables, Industrial and Medicinal Plants, Roodeplaat Campus, South Africa.
- **Ms Karen De Jager:** ARC-Tropical & Subtropical Crops, Mbombela Campus, South Africa
- **Prof. Lyndy J. McGaw:** University of Pretoria, Onderstepoort Campus, South Africa
- **Dr Tsholofelo Mokoka:** University of Pretoria, Onderstepoort Campus, South Africa
- **Mr Jermiah Senabe:** University of Johannesburg, Doornfontein Campus, South Africa
- **Prof Zakheleni Dube:** University of Mpumalanga, Mbombela Campus, South Africa.
- **Prof. Pathu. W. Mashela:** University of Limpopo, Turfloop Campus, South Africa.
- **Prof. Ashwell Ndhlala:** University of Limpopo, Turfloop Campus, South Africa.
- **Ms Karen Swanepoel:** The Southern African Essential Oil Producers' Association (SAEOPA), Pretoria, South Africa.
- **Dr Buntubonke Gom**: Council for Scientific and Industrial Research (CSIR), Pretoria Campus, South Africa.

THANK YOU