Effect of *Maerua angolensis* and *Tabernaemontana elegans* leaf meal extracts on *Meloidogyne incognita* second-stage juvenile hatch and viability

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Summary. The study examines the nematicidal effect of five leaf-meal extracts of *Maerua angolensis* and *Tabernaemontana elegans* on hatching and viability of second-stage juveniles (J2) of *Meloidogyne incognita* in *in vitro* assays. At all exposure times, J2-hatch inhibition and mortality caused by all plant extracts was significantly higher compared with the pluronic gel + deionised H_2O treatment. Percentage J2-hatch inhibition and mortality increased with increasing exposure time for all extracts of both plants. After 21-days exposure, the partitioned-deionised H_2O extracts of *M. angolensis* and *T. elegans* caused the highest J2 hatch inhibition (65.5 and 62.7%, respectively). After 72 h exposure, the partitioned-DCM and MeOH/DCM extracts of *M. angolensis* and the partitioned-deionised H_2O extract of *T. elegans* had caused the highest J2 mortality (49.7, 48.1 and 48.3%, respectively). The results suggest that the compound of *T. elegans* causing J2 mortality may be either different in type or concentration to those in *M. angolensis*.

Key words: medicinal plants, phytonematicides, root-knot nematode, salicylic acid.

During the past two decades, phytonematicides (botanical nematicides, bionematicides), secondary metabolites produced by plants, have become a desirable component of nematode management technology and practices (Chitwood, 2002; Ntalli & Caboni, 2012; Caboni & Ntalli, 2014; Mashela *et al.*, 2017). The basic materials for the development of phytonematicides are often collected from locally available plants (*e.g.*, Akhtar, 2000; Ibrahim *et al.*, 2006; Slomp *et al.*, 2009; Ntalli & Caboni, 2012; Mashela *et al.*, 2017; Hernández-Carlos & Gamboa-Angulo, 2019).

Research on the nematicidal or nematostatic effect of plant extracts and their nematode-suppressive compounds has mainly concentrated on root-knot nematodes (*Meloidogyne* spp.) and has led to a wealth of information on substances of plant origin that are toxic to this group of plant-parasitic nematodes, especially *Meloidogyne incognita* (*e.g.*, Akhtar & Mahmood, 1994; Ibrahim *et al.*, 2006; Slomp *et al.*, 2009; Hong *et al.*, 2010, 2011; Odeyemi & Adewale, 2011; Ntalli & Caboni, 2012; Ullah *et al.*, 2014; Dube & Mashela, 2016, 2017; Ntalli *et al.*, 2016; Regaieg *et al.*, 2017; Akhter *et al.*, 2018; Oplos *et al.*, 2018; Tarraf *et al.*, 2019; de Freitas Silva *et al.*, 2020). Root-knot nematodes are considered worldwide one of the economically most important groups of plant-parasitic nematodes

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(Moens *et al.*, 2009; Jones *et al.*, 2013). Nematode-suppressive compounds can either repel or inhibit the infective second-stage juvenile (J2) to reach the roots or, when J2 have penetrated the roots, inhibit their movement or development inside the roots, even causing death of the nematodes (Wuyts *et al.*, 2006). Most research has focused on J2 hatch inhibition and direct-contact J2 motility and or viability (*e.g.*, Hong *et al.*, 2010, 2011; Adomako & Kwosheh, 2013; Ullah *et al.*, 2014; Ntalli *et al.*, 2016; Tarraf *et al.*, 2019).

In South Africa (SA), so-called 'muti' are traditional medicines that make use of various natural products derived from trees and other plant species that are mostly endemic to Southern Africa. These botanical medicines are usually prescribed by herbal healers to treat humans and domestic animals for various ailments (Van Wyk *et al.*, 2009). Recently in a series of field trials, Khosa *et al.* (2020a) examined the root-knot nematode-suppressive and plant growth-stimulating potential of crudely milled powders of stems, leaves and bulbs of several plant species of which natural products are being used as muti in traditional medicine in SA. Relative to the non-treated control plants, soil amendments of *Maerua angolensis* DC. (bead-been tree) and *Tabernaemontana elegans* Stapf (toad tree), two traditional medicines that are easy to obtain locally in SA, consistently suppressed the population densities of *M. incognita* in tomato roots. Using the curve-fitting allelochemical response dosage (CARD) model, Khosa *et al.* (2020b) showed the low minimum concentrations for *M. incognita* J2 hatch inhibition and lethal concentrations of *M. angolensis* and *T. elegans* plant extracts (leaf meals), and moderate sensitivity values provided substantial evidence of the high potency of the two plant extracts in the potential management of *M. incognita*.

In the present study, the effect of five different leaf meal extracts of *M. angolensis* and *T. elegans* on the hatching and viability of J2 of *M. incognita* race 2 in *in vitro* assays was examined to identify the extraction method that resulted in the highest nematicidal effect, and to further compare the nematicidal effects and characterise the nematode-suppressive compound(s) of these two plant species.

MATERIAL AND METHODS

The *in vitro* assays were carried out in the laboratory of the Biosciences Unit at the campus of the Council for Scientific and Industrial Research (CSIR), Tshwane, Gauteng Province, SA.

Plant material and preparation of powdered leaf meals. Leaves of *M. angolensis* and *T. elegans* were collected from traditional healers in the Limpopo Province, SA. The botanical origin of the leaf samples was identified and confirmed by a botanist from the South African National Biodiversity Institute (SANBI, Tshwane, Gauteng Province, SA). The leaves were chopped into 5-cm-long pieces and oven-dried for 4 days at 52°C prior to grinding in a Wiley mill and passing through a 1-mm-aperture sieve. Twenty-five kg of the crudely-milled, powdered leaf meals of each plant species were stored in bulk in marked, air-tight glass containers at room temperature in the dark until they were subjected to the procedure to obtain the different extracts used in the study.

Extraction procedure (Fig. 1). One kg of the powdered leaf meals of each plant species was soaked in 1 l deionised H_2O for 24 h at room temperature, filtered and the filtrate freeze-dried to obtain the crude extract (treatment A). Another 1 kg of the powdered leaf meals of each plant species was shaken in a methanol (MeOH)/dichloromethane (DCM; 1:1 v:v) solution for 8 h. This process was repeated after which the residue was discarded and the liquid evaporated at 60°C to obtain the MeOH/DCM extract (treatment B). Fifteen g of the MeOH/DCM extract was then partitioned in a solution of 50% hexane + 45% MeOH + 5% H_2O. After 24 h, two layers had formed, namely the hexane layer and the aqueous MeOH. The hexane layer on top was poured off and evaporated at 60°C to obtain the hexane extract (treatment C). The aqueous MeOH layer at the bottom was evaporated at 60°C and further partitioned in a DCM/deionised H_2O (1:1 v:v) solution for 25 min. The H_2O layer on top was poured off and freeze-dried to obtain the partitioned-deionised H_2O extract (treatment D). The DCM layer at the bottom was evaporated at 60°C to obtain the partitioned-DCM extract (treatment E).

Preparation of stock solutions. Five stock solutions were prepared for each of the five plant extracts. Using an analytical balance, 10 mg aliquots of each extract were added to 1.5 ml Eppendorf tubes. The freeze-dried crude extract (treatment A) and partitioned-deionised extract (treatment D) were dissolved in a solution of Pluronic gel + deionised H_2O up to the 1 ml mark of each Eppendorf tube. The dried MeOH/DCM (treatment B), hexane (treatment C) and partitioned-DCM (treatment E) extracts were dissolved in 10% MeOH up to the 1 ml mark of each Eppendorf tube. The tubes were closed tightly and the extracts brought into suspension

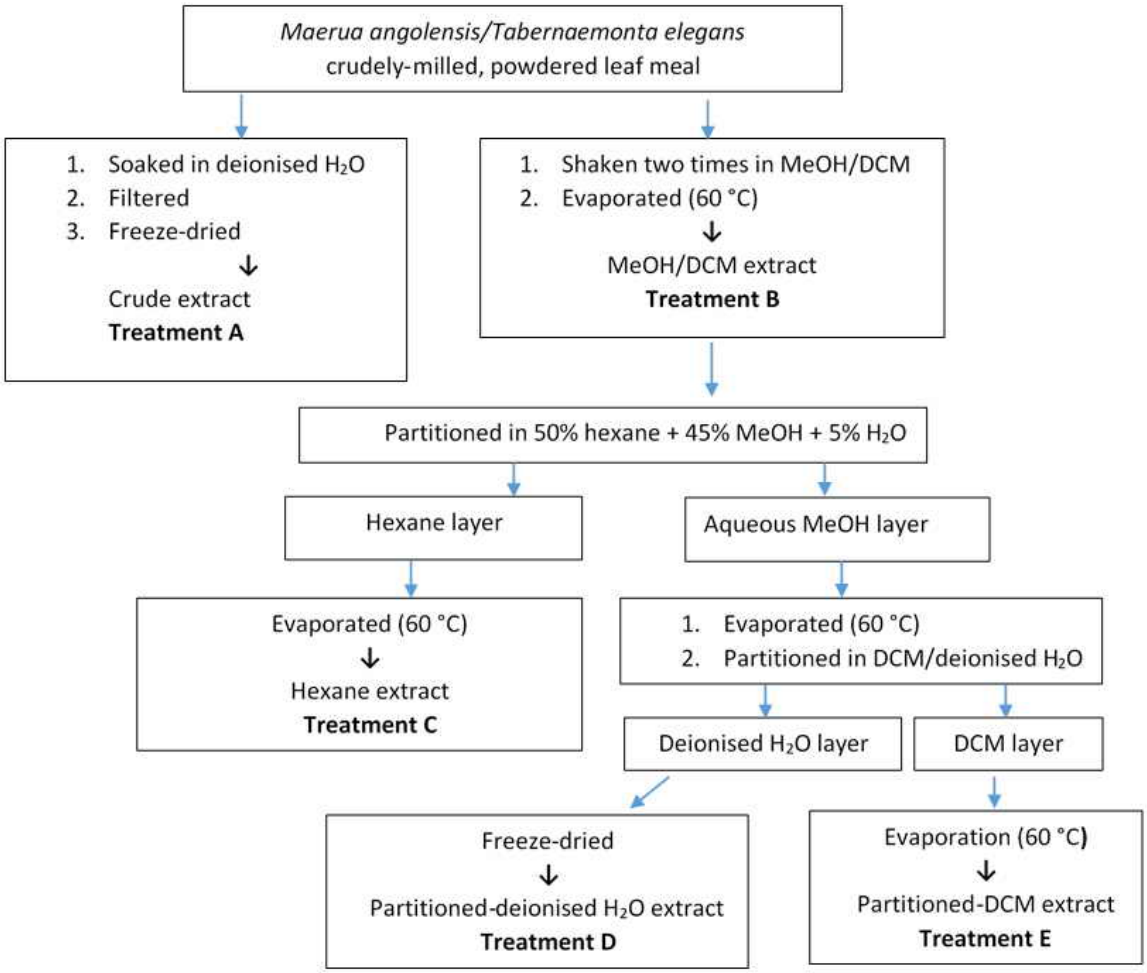

Fig. 1. Schema of the extraction methods used to obtain the five extracts of the leaf meals of *Maerua angolensis* and *Tabernaemonta elegans*. MeOH: methanol; DCM: dichloromethane.

in each tube by thorough shaking for 45 min. The stock solutions were stored at –4°C in a deep freezer to maintain their stability.

Preparation of nematode inoculum. A population of *M. incognita* race 2, of which the identification was confirmed by sequence-characterised amplified regions-polymerase chain reaction (SCAR-PCR) (Zijlstra *et al.*, 2000; Fourie *et al.*, 2001), was obtained from the ARC-Grain Crops Institute, Potchefstroom, SA, and multiplied during 2 months in a glasshouse on plants of the susceptible tomato 'Floradade'. Egg masses obtained from infected tomato plants were shaken in a 1% NaOCl solution for 30 s to remove the gelatinous matrix surrounding the eggs and surface-sterilise the eggs (Riekert, 1995). The eggs were then thoroughly rinsed in distilled water before used in the J2 hatch assays. Freshly-hatched J2 were obtained by transferring the surface-sterilised eggs in Petri dishes containing 10 ml distilled water and placed in an incubator at 25 ± 2°C. J2 that had hatched during the first 24 h were discarded. J2 that had hatched during the subsequent 48 h were used in the viability assays (Dube & Mashela, 2016).

Effect on J2 hatch. The effect of a 10% dilution of each of the five plant extracts on the hatching of J2 was tested in 96-well plates. A 90 µl suspension of distilled water containing 100 ± 20 freshly-collected eggs was added to each well containing 90 µl of diluted extract (*i.e.*, a concentration of 1 mg ml^{-1}). The well plates were sealed with parafilm, shaken for 10 min at 1,000 rpm using a laboratory shaker and placed in an incubator at 22 ± 2°C in the dark. The treatments were assigned to the wells in a completely randomised design with four wells representing four replications per treatment. The number of J2 that had hatched after 7, 14 and 21 days exposure to the extracts were counted using a stereomicroscope. The assay was repeated once. Three controls were included in the

Table 1. Effect of five extracts of leaf meals of *Maerua angolensis* and *Tabernaemontana elegans* and three control treatments after 7, 14 and 21 days exposure on hatching of second-stage juveniles (J2) of *Meloidogyne incognita* race 2 under *in vitro* conditions.

Treatment	Extract	J2 hatch inhibition (%)					
		Maerua angolensis			*Tabernaemontana elegans*		
		7 days	14 days	21 days	7 days	14 days	21 days
A	Crude extract	9.7 cd	41.1 c	60.5 c	15.9 bc	44.0 c	59.2 c
B	Methanol/dichloromethane (MeOH/DCM) extract	9.3 d	38.8 d	56.1 d	15.9 bc	40.2 d	55.7 d
C	Hexane extract	10.1 bc	36.9 e	54.4 d	16.5 b	44.6 c	59.2 c
D	Partitioned-deionised H_2O extract	11.0 a	46.9 b	65.5 b	18.2 a	47.1 b	62.7 ab
E	Partitioned-dichloromethane (DCM) extract	10.4 ab	36.4 e	46.2 e	13.3 d	42.7 d	54.4 e
F	Pluronic gel + deionised H_2O	1.3 g	3.0 f	3.1 f	4.4 e	4.4 e	6.9 f
G	10% methanol (MeOH)	5.1 f	45.7 b	70.5 a	14.5 cd	44.5 c	61.2 bc
H	Salicylic acid	8.6 e	49.6 a	73.4 a	16.2 b	50.9 a	65.3 a
	$LSD_{0.05}$	0.0318	0.0178	0.0179	0.0279	0.0203	0.0216
	P-value	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	F-value	553.68	3,912.71	5,048.36	111.82	186.02	99.82

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.

assays: a solution of pluronic gel + deionised H_2O, 10% MeOH, and 1.8 mg ml^{-1} salicylic acid.

Effect on J2 mortality. The effect of a 10% dilution of each of the five plant extracts on the mortality of the J2 was tested in 96-well plates. A 90 µl suspension of distilled water containing 100 ± 20 freshly-collected J2 was added to each well containing 90 µl of diluted extract (*i.e.*, a concentration of 1 mg ml^{-1}). The well plates were sealed with parafilm, shaken for 10 min at 1,000 rpm using a laboratory shaker and placed in an incubator at 22 ± 2°C in the dark. The treatments were assigned to the wells in a completely randomised design with four wells representing four replications per treatment. The number of J2 that were dead after 24, 48 and 72 h exposure to the extract were counted using a stereomicroscope. The J2 were considered dead when no movement was observed during 2 s even after prodding with a needle. The assay was repeated once. Three controls were included in the assays: a solution of pluronic gel + deionised H_2O, 10% MeOH, and 1.8 mg ml^{-1} salicylic acid. Pluronic gel is a clear, non-ionic and non-toxic co-polymer gel; its low density allows J2 to move freely inside the gel and to be observed easily using a stereomicroscope. Pluronic gel + deionised H_2O and 10% MeOH were included as controls because they were used to prepare the extracts; salicylic acid was included as a control because its effect on hatching and motility inhibition of J2 of *M. incognita* over time has been well documented by Wuyts *et al.* (2006).

Statistical analysis. Since no significant differences were observed between the two runs of each of the two assays, the results were pooled. The percentage of J2 that had either hatched or were dead after each exposure period were arcsine transformed to homogenise variances (Gomez & Gomez, 1984) prior to analysis of variance using SAS 9.2 statistical software (SAS Institute, 2008). Treatment means were separated using Fisher's protected least significant difference (LSD) test ($P \leq 0.05$; Snedecor & Cochran, 1980).

RESULTS

Effect on J2 hatch. Percentage J2 hatch inhibition increased with increasing exposure time for all plant extracts: from 9.3-11.0% after 7 days exposure to 46.2-65.5% after 21 days exposure (*M. angolensis* extracts) and from 13.3-18.2% after 7 days exposure to 54.4-62.7% after 21 days exposure (*T. elegans* extracts; Table 1). At all exposure times, all extracts of both *M. angolensis* and *T. elegans* resulted in a significant ($P \leq 0.05$) increase in J2 hatch inhibition compared with the pluronic gel + deionised H_2O treatment. After 7 days exposure, all extracts of *M. angolensis* had also caused a significantly ($P \leq 0.05$) higher J2 hatch inhibition compared with the 10% MeOH and salicylic acid treatments but after 14 and 21 days exposure, J2 hatch inhibition caused by the *M. angolensis* extracts was significantly ($P \leq 0.05$) lower compared with the 10% MeOH and salicylic

acid treatments, with one exception (partitioned-DCM extract). After 7 days exposure, only one of the extracts of *T. elegans* (partitioned-deionised H_2O extract) had caused a significantly ($P \leq 0.05$) higher J2 hatch inhibition compared with the 10% MeOH and salicylic acid treatments, while J2 hatch inhibition caused by the other four extracts of *T. elegans* was not significantly different either from the 10% MeOH or the salicylic acid treatment. After 14 and 21 days exposure, J2 hatch inhibition caused by three of the five extracts of *T. elegans* was significantly ($P \leq 0.05$) lower compared with the salicylic treatment but not significantly different compared with the 10% MeOH treatment, while J2 hatch inhibition caused by the other two extracts of *T. elegans* was also significantly ($P \leq 0.05$) lower compared with the 10% MeOH treatment. At all exposure times, significant ($P \leq 0.05$) differences in percentage J2 hatch inhibition were observed among the extracts of both *M. angolensis* and *T. elegans*: after 21 days exposure, the partitioned-deionised H_2O extracts of *M. angolensis* and *T. elegans* had caused the highest ($P \leq 0.05$) J2 hatch inhibition, while the partitioned-DCM extracts of *M. angolensis* and *T. elegans* had caused the lowest ($P \leq 0.05$) J2 hatch inhibition.

Effect on J2 mortality. Percentage J2 mortality increased with increasing exposure time for all extracts of *M. angolensis*: from 13.1-19.8% after 24 h exposure to 38.1-49.7% after 72 h exposure (Table 2). Percentage J2 mortality increased with increasing exposure time for three of the extracts of *T. elegans*: from 18.0-21.6% after 24 h exposure to 32.1-48.3% after 72 h exposure. The hexane and partitioned-DCM extracts of *T. elegans* also caused an increase in J2 mortality after 48 h exposure compared with 24 h exposure (from 6.3-7.0% to 14.7-17.0%) but no more increase in J2 mortality was observed after 72 h exposure. At all exposure times, all extracts of both *M. angolensis* and *T. elegans* resulted in a significant ($P \leq 0.05$) increase in J2 mortality compared with the pluronic gel + deionised H_2O treatment. At all exposure times, all extracts of *M. angolensis* and *T. elegans* caused a J2 mortality that was significantly ($P \leq 0.05$) higher compared with the 10% MeOH treatment but significantly ($P \leq 0.05$) lower compared with the salicylic acid treatment. At all exposure times, significant ($P \leq 0.05$) differences in percentage J2 mortality were observed among the extracts of both *M. angolensis* and *T. elegans*. After 72 h exposure, the MeOH/DCM and partitioned-DCM extracts of *M. angolensis* and the partitioned-deionised H_2O extract of *T. elegans* had caused the highest ($P \leq 0.05$) J2 mortality, while the hexane extract of *M. angolensis* and the partitioned-DCM extract of *T. elegans* had caused the lowest ($P \leq 0.05$) J2 mortality.

Table 2. Effect of five extracts of leaf meals of *Maerua angolensis* and *Tabernaemontana elegans* and three control treatments after 24, 48 and 72 hours (h) exposure on viability of second-stage juveniles (J2) of *Meloidogyne incognita* race 2 under *in vitro* conditions.

Treatment	Extract	J2 mortality (%)					
		Maerua angolensis			*Tabernaemontana elegans*		
		24 h	48 h	72 h	24 h	48 h	72 h
A	Crude extract	16.3 e	30.7 e	43.6 d	19.8 b	26.7 d	37.9 c
B	Methanol/dichloromethane (MeOH/DCM) extract	19.8 b	43.3 b	48.1 b	18.0 b	29.0 c	32.1 d
C	Hexane extract	16.7 c	31.2 d	38.1 e	7.0 d	17.0 e	17.1 e
D	Partitioned-deionised H_2O extract	13.1 d	42.1 c	47.2 c	21.6 c	43.3 b	48.3 b
E	Partitioned-dichloromethane (DCM) extract	15.2 c	41.0 c	49.7 b	6.3 e	14.7 f	16.3 f
F	Pluronic gel + deionised H_2O	0.0 f	0.5 f	1.2 g	0.1 g	0.6 h	2.2 h
G	10% methanol (MeOH)	0.1 f	0.6 f	1.6 f	7.8 f	9.9 g	10.5 g
H	Salicylic acid	86.5 a	96.0 a	95.3 a	81.2 a	86.0 a	85.5 a
	$LSD_{0.05}$	0.0381	0.0269	0.0219	0.0407	0.0305	0.0250
	P-value	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	F-value	2,062.25	5,203.36	7,419.14	1,515.53	2,742.51	4,199.27

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.

DISCUSSION

The results of our study confirm the report by Khosa *et al.* (2020b) that extracts of both *M. angolensis* and *T. elegans* suppress hatching and cause mortality of hatched J2 of *M. incognita* race 2 under *in vitro* conditions. These effects on nematode development explain, at least partly because other suppressive effects cannot be excluded, the nematode-suppressive effects of crudely milled powders of plant parts of *M. angolensis* and *T. elegans* on *M. incognita* race 2 population densities in the roots of tomato plants under glasshouse conditions (Khosa *et al.*, 2020a). Hatch inhibition and mortality of J2 of *M. incognita* are nematode-suppressive effects that have often been reported when botanical nematicides are examined under *in vitro* conditions (*e.g.*, Hong *et al.*, 2010, 2011; Adomako & Kwosheh, 2013; Ullah *et al.*, 2014; Ntalli *et al.*, 2016; Oplos *et al.*, 2018; Tarraf *et al.*, 2019).

In general, the effect of the extracts of *M. angolensis* and *T. elegans* on J2 hatch when compared after 14 and 21 days exposure was comparable, ranging from 36.9 to 46.9% and 46.2 to 65.5%, respectively (*M. angolensis*) and from 40.2 to 47.1% and 54.4 to 62.7%, respectively (*T. elegans*). After 7 days exposure, the extracts of *T. elegans* caused a somewhat higher J2 hatch inhibition compared with *M. angolensis* (from 13.3 to 18.2% *vs* 9.3 to 11.0%) but this difference may be due to the 'background' J2 hatch inhibition (pluronic gel + deionised H_2O treatment), which was somewhat higher for the *T. elegans* assay compared with the *M. angolensis* assay (4.4 *vs* 1.3). At all exposure times, the partitioned-deionised H_2O extract of both *M. angolensis* and *T. elegans* caused significantly the highest J2 hatch inhibition compared with the other four plant extracts with only one exception (partitioned-DCM extract of *M. angolensis* after 7 days exposure). After 7 days exposure, the J2 hatch inhibition caused by the partitioned-deionised H_2O extracts of both *M. angolensis* and *T. elegans* was significantly higher compared with the salicylic acid treatment but this was not the case anymore after 14 and 21 days exposure when the salicylic acid treatment caused a significantly higher J2 hatch inhibition compared with the partitioned-deionised H_2O extract of both *M. angolensis* and *T. elegans*.

In general, the effect of three of the extracts of *T. elegans* (crude, MeOH/DCM and portioned-deionised H_2O extracts) and all five extracts of *M. angolensis* on J2 mortality was comparable at all exposure times, ranging from 13.1 to 19.8% and 18.0 to 21.6%, respectively, after 24 h exposure, from 30.7 to 43.3% and 26.7 to 43.3%, respectively, after 48 h exposure, and from 38.1 to 49.7% and 32.1 to 48.3%, respectively, after 72 h exposure. The observation that the effects of the hexane and partitioned-DCM extracts of *T. elegans* relative to *M. angolensis* were at all exposure times consistently significantly lower (from 45.6 to 67.3%) compared with the effects of the same extracts of *M. angolensis* suggests that the compound of *T. elegans* causing J2 mortality may be present in either a different concentration in *T. elegans* than in *M. angolensis* or may be different from the compound causing J2 mortality in *M. angolensis*. The effect on J2 mortality differed somewhat among the five extracts of *M. angolensis* at each exposure time but after 48 and 72 h exposure the partitioned-DCM, MeOH/DCM and partitioned-deionised H_2O extracts of *M. angolensis* caused significantly the highest J2 mortality (between 40 and 50%) compared with the crude and hexane extracts. By contrast, the partitioned-deionised H_2O extract of *T. elegans* caused consistently the highest J2 mortality, while the hexane and partitioned-DCM extracts caused consistently the lowest J2 mortality at all exposure times. This observation re-enforces the suggestion made above that the compound of *T. elegans* causing J2 mortality may be present in either a different concentration than in *M. angolensis* or may be different from the compound causing J2 mortality in *M. angolensis*.

Wuyts *et al.* (2006) reported that, at the concentrations used in their study, salicylic acid reduced hatching of J2 of *M. incognita* by 27% (after 10 days exposure) and gave a 100% motility inhibition of the J2 after 72 h exposure. They noted that the concentration of salicylic acid to inhibit hatch by 50% was about 10 times higher than the concentration to inhibit motility and kill 50% of the J2. The results of our study are in agreement with these observations: the salicylic acid treatment reduced a J2 hatch inhibition of 8.6 and 49.6% after 7 and 14 days, respectively, in the *M. angolensis* assay and of 16.2 and 50.9% after 7 and 14 days, respectively, in the *T. elegans* assay; the salicylic acid treatment resulted in a J2 mortality higher than 80% after 24 h exposure in both *M. angolensis* and *T. elegans* assays, and 95.3 and 85.5% after 72 h in the *M. angolensis* and *T. elegans* assays, respectively. Drench and foliar applications of salicylic acid have been effective in suppressing *M. incognita* population densities (Maheshwari & Anwar, 1990; Nandi *et al*., 2003).

The present study identifies the extraction methods that result in the extracts with the highest

nematicidal effect. The data suggest that the compound(s) of *T. elegans* causing J2 mortality may be present in either a different concentration in *T. elegans* than in *M. angolensis* or may be different from the compound(s) causing J2 mortality in *M. angolensis*. Further study is necessary to identify the phytochemicals that are causing the observed nematicidal effects.

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M.C. Khosa, Z. Dube, M. Tselanyane, J. Senabe, G. Fouche, D. De Waele and M.S. Daneel. Воздействие экстрактов листьев *Maerua angolensis* и *Tabernaemontana elegans* на вылупление и жизнеспособность личинок второй стадии *Meloidogyne incognita*.

Резюме. Исследовано нематицидное действие пяти вариантов экстракта листьев растений *Maerua angolensis* и *Tabernaemontana elegans* на вылупление и жизнеспособность личинок второй стадии (J2) *Meloidogyne incognita* в экспериментах *in vitro*. При всех продолжительностях воздействия экстрактами этих растений, подавление вылупления J2 и их смертность были выше, чем в контроле – при обработке гелем на основе плюроника (полоксамера) на деионизированной воде. Доля погибших личинок и J2 с остановленным вылуплением возрастала с увеличением времени экспозиции для всех полученных экстрактов от этих растений. После 21-дневной экспозиции, фракции экстрактов *M. angolensis* и *T. elegans*, полученные в деионизированной воде, вызывали наибольшее подавление вылупления J2 (65.5 и 62.7%, соответственно). После 72-часовой экспозиции, фракция экстракта из *M. angolensis*, выделенная с помощью дихлорметана, и фракция, выделенная с помощью метанола и дихлорметана из этого же растения, а также экстрагированная с помощью деионизированной воды фракция экстракта *T. elegans* давали наивысшую смертность J2 (49.7, 48.1 и 48.3%, соответственно). Полученные результаты позволяют предположить, что выделяемое из *T. elegans* соединение, вызывающее смертность J2, отличается по своей химической структуре или по получаемой концентрации от активного соединения, получаемого из *M. angolensis*.