

## POTENTIAL USE OF ENVIRONMENT-FRIENDLY *MAERUA ANGOLENSIS* EXTRACTS AS ALTERNATIVE TO SYNTHETIC NEMATOCIDES IN RURAL SMALLSCALE CASSAVA PRODUCTION

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### Abstract

*Cassava is a crop with great potential in addressing the challenges of malnutrition, rampant in the African continent. In southern Africa, the crop has a minor status being produced mainly by smallholder farmers until recently. There is scant information on the effects of root-knot nematodes in cassava production. The aim of the study was to determine the contribution of Maerua angolensis extracts in the management of Meloidogyne incognita in two cassava cultivars (cv. 'Mbonisweni' and 'Mganduzweni'). Treatments were laid-out in a randomised complete block design (RCBD) under greenhouse conditions with five replications. Treatments consisted of 0, Nematicur, 184; 368; 736; 1472; and 2944 kg/ha of M. angolensis. Both cultivars had nematode reproductive-factors and potential greater than one and no nematode effect were observed on plant growth of the two cassava cultivars. M. angolensis had equivalent effect on M. incognita populations as commercial nematicide, Nematicur. In conclusion, 'Mbonisweni' and 'Mganduzweni' cultivars were tolerant to M. incognita and M. angolensis plant extract have potential use as an alternative to commercial nematicides in cassava production.*

**Key words:** cassava, host-status, *Meloidogyne incognita*, *Maerua angolensis*, susceptibility.

### INTRODUCTION

Cassava cv. 'Mbonisweni' has been reported to be tolerant to *M. incognita* (Timana et al., 2021). Since the crop is mainly grown in subsistence farming as an intercrop, the build-up of nematodes in the presence of the crop may affect the susceptible crops in the intercrop or a rotation with a susceptible crop (Kimaru, 2013). With the limited use of synthetic chemicals in subsistence farming, any build-up of nematodes is not desired as it creates a challenge for the next crop in rotation or an intercrop (Talwana et al., 2015). Currently, there are no reports of nematode management in the subsistence cassava farming sector, yet in the commercial sector, the management has relied mostly on the use of synthetic chemicals nematicides (Sithole et al., 2021).

Plant parasitic nematodes are thought to be responsible for 12.3% of agricultural losses, or USD 157 billion each year (Mendoza-de Gives,

2022). The environmental problems associated with the use of synthetic chemicals includes greenhouse gas pollution, mutagenicity, soil degradation, and depletion of aquatic life (Sithole et al., 2021). Some of the human health issues associated with synthetic chemicals include cancer, human endocrine disruption, infertility, and headaches (Jallow et al., 2017; Talwana et al., 2015). The use of synthetic nematicides in the subsistence farming sector is also challenged by the size of land under production, which affects the economies of scale and the knowledge of handling hazardous substances (Talwana et al., 2015). Misuse, health complications, and improper handling of synthetic chemicals especially in subsistence farming have been reported, with some smallholder farmers in Tanzania reported to be applying pesticides weekly in their farms without consideration of the pest injury levels (Talwana et al., 2015). In India, sprayers, and farmworkers exposed to chemicals such as

methyl parathion were reported to suffer from cardiotoxic effects (Aktar et al., 2009).

Different plant extracts have shown the ability to manage root-knot nematode population in different crops over the years (Khosa et al., 2020a; Akpheokhai et al., 2012; Taye et al., 2012). Some of the widely assessed plant extracts include *Tagetes* species (Kalaiselvam & Devaraj, 2011), *Cucurbita maxima*, *Tithonia diversifolia*, *Azadirachta indica*, *Zanthoxylum zanthoxyloides* and *Datura metel* (Akpheokhai et al., 2012), and *Inula viscosa* (Ibrahim et al., 2016). *Maerua angolensis* and *Tabernaemontana elegans* have also suppressed *M. incognita* populations in tomato plants under glasshouse and field conditions (Khosa et al., 2020a). Two major disadvantages in the use of plant extracts have been the inconsistencies in the performance and phytotoxicity (Mashela et al., 2015). Mashela et al. (2015) postulated that these challenges are mainly caused by the use of too low or high dosages, affecting the level of adoption of plant extracts in several crops, in several countries.

The extensive work that has been done on the nematocidal effect of plant extracts with their root-knot nematode suppressive compounds, has led to the discovery of plant substances that are toxic to *M. incognita* (Khosa et al., 2021). These plant extracts with the ability to suppress root-knot nematode can either inhibit or repel second-stage juveniles to penetrate plant roots or restrict the movement of J2 after penetrating the root system, ultimately killing the nematode (Khosa et al., 2021). *Maerua angolensis* has been reported to have suppressed *M. incognita* populations in tomato plants under glasshouse and field conditions (Khosa et al., 2020a), but this has not been confirmed in cassava plants. Therefore, the present study aims to establish the potential of *M. angolensis* plant extracts in suppressing *M. incognita* and promoting growth in two locally produced cassava cultivars, cv. 'Mbonisweni' and cv. 'Mganduzweni'.

## MATERIALS AND METHODS

### Study area

The study was conducted at the University of Mpumalanga greenhouse (25°43'64'' S, 30°98'17'' E), Mbombela, Mpumalanga, South Africa with controlled temperatures of 25±5 °C.

### Medicinal plant material collection and extract preparation

Stems and leaves of *M. angolensis* were collected from the Agricultural Research Council, Nelspruit. The botanical origin of the plants was identified and verified by a botanist from the South African National Biodiversity Institute (SANBI, Tswane), and the material were stored as code 3112000/PRE099594-0 (Khosa et al., 2020a). The *M. angolensis* plant parts were cut into 5 cm long parts and dried using an oven set at 52 °C for 4 days before being ground using Wiley mill and passed through a 1 mm sieve (Khosa et al., 2020a). The crudely milled plant material was kept in marked, air-tight glass vessels at 25°C in the shade until required for usage.

### Preparation of nematode inoculum

Populations of *M. incognita* were acquired from the Agricultural Research Council, Nelspruit. The identity of *M. incognita* was confirmed using sequence-characterized amplified regions-polymerase chain reaction (SCAR-PCR) (Khosa et al., 2021). *Meloidogyne incognita* populations were multiplied by introducing a sample of confirmed nematodes to susceptible tomato cv. 'Floradade' for two months. Nematode eggs and J2 used were extracted from roots of tomato plants by blending and maceration of plant roots in a 1% NaCl solution (Marais et al., 2017). The aliquot was then sieved through several nested sieves with different apertures: 150, 63, and 25 µm, with inoculum collected from the smallest sieve.

### Treatments and experimental design

Cassava sprouts of cv. 'Mbonisweni' and 'Mganduzweni' were separately exposed to *M. angolensis* powder levels of 0, 184, 368, 736, 1 472, and 2 944 kg of extract/ha with five replications in a randomised complete block design. Untreated cassava plants were used as negative control while plants treated with Nemacur® (400 g fenamiphos/L) were used as a positive control across all replications. In all replications, a nematode susceptible tomato cv 'Floradade' was included as an indicator of nematode viability.

### Data collection

Plant variables: At 72 days after inoculation, the length of sprouts were measured from the basal part to the end of the flag leaf using a 30 cm

ruler. Chlorophyll content was measured from the topmost matured leaves using a chlorophyll meter (Spad-502, Minolta, Japan). Sprouts were removed from cutting buds and stem diameter measured 5 cm from the distal end of the cut shoot using a Vernier caliper (GV9370, Grip, Johannesburg). The shoots were then dried in an oven set at 52°C for 72 hours and weighed (Timana et al., 2021).

Nematode variables: Using the blending and maceration method, nematodes were extracted from the total root system per plant (Marais et al., 2017). The sugar-floatation and centrifugation methods were used to extract J2 from soil samples (Marais et al., 2017). Eggs and J2 from cassava roots and soil samples were separately counted from a 1-ml aliquot under a stereomicroscope (Model CX23RTFS2, Olympus Corporation, Tokyo) at X40 magnification. Nematode numbers per 1 ml aliquot were extrapolated to nematodes/total root system, while nematode numbers from the soil were extrapolated to total nematodes per total volume of growing media, all to allow for the determination of final nematode population density (Pf).

Reproductive potential (RP) and reproductive factor (RF) were then computed (Sasser, 1984):

$$RP = \frac{\text{Total nematode population}}{\text{Fresh root mass}}$$

$$RF = \frac{\text{Final population per pot}}{\text{Initial population per pot}}$$

### Data analysis

The nematode and plant growth variable data were subjected to analysis of variance (ANOVA) through Statistix10 software. Before

ANOVA, Shapiro-Wilk's normality test was used to test for deviation from normality in each standardised residuals variable (Gomez & Gomez, 1983). Inherent variabilities were removed by subjecting data to a  $\text{Log}_{10}(x+1)$  transformation. Mean separation was achieved using Fisher's least significant difference at 5% probability level.

## RESULTS AND DISCUSSIONS

Shapiro-Wilk normality tests indicated that all nematode and plant growth variables were not normally distributed ( $P \leq 0.05$ ), except for chlorophyll content, thus measured variables were transformed accordingly. The cassava root systems were mostly clean of nematode galls, with visible ones being extremely small and underdeveloped.

Plant extract level effects were highly significant ( $P \leq 0.01$ ) for all nematode variables measured including the reproductive factor and potential except for number of eggs in roots ( $P \leq 0.05$ ), while in all plant growth variables there were no statistically significant effects, except for number sprouts, dry shoot mass, plant height and stem diameter (Tables 1 and 2). The reproductive factor were greater than 1 across all the plant extract levels except for at 2 g, which were not different from both the negative and positive control. The reproductive potential was greater than 1 across all plant extract levels except for positive control (Table 1). Across all nematode variables, there were more nematode populations in plants treated with *M. angolensis* except at 6 g, which was not different from both controls. The plant extract generally improved the plant growth variables of cv. 'Mbonisweni' better than 'Mganduzweni' (Table 2).

Table 1. Effect of *Maerua angolensis* application quantity on nematode variables, reproductive factor, potential and stem diameter

Crude extract level	Juveniles in roots	Total nematodes in roots	Stem diameter	Juveniles in soil	Total nematodes in a pot	Reproductive Factor	Reproductive Potential
0	0.9144 <sup>bc</sup> (570.00)	1.1785 <sup>b</sup> (520.00)	0.1639 <sup>c</sup> (0,4610)	1.3208 <sup>bcd</sup> (7600.0)	1.9105 <sup>c</sup> (8120.0)	0.3254 <sup>bc</sup> (2.7067)	0.8169 <sup>b</sup> (62.888)
2	1.3857 <sup>ab</sup> (143.23)	1.4423 <sup>ab</sup> (192.06)	0.1805 <sup>bc</sup> (0,5202)	0.8486 <sup>cd</sup> (2508.2)	2.0151 <sup>bc</sup> (2700.3)	0.1702 <sup>c</sup> (0.9001)	0.8758 <sup>b</sup> (18.877)
4	1.5415 <sup>ab</sup> (570.00)	1.6878 <sup>ab</sup> (660.00)	0.2024 <sup>ab</sup> (0,5960)	2.7947 <sup>ab</sup> (9200.0)	3.7324 <sup>a</sup> (9860.0)	0.5191 <sup>ab</sup> (3.2867)	1.1224 <sup>ab</sup> (88.407)
6	0.5944 <sup>bc</sup> (136.33)	0.9766 <sup>bc</sup> (177.64)	0.2198 <sup>a</sup> (0,6584)	2.5112 <sup>abc</sup> (9962.2)	2.8187 <sup>abc</sup> (10140)	0.4444 <sup>ab</sup> (3.3799)	0.5926 <sup>bc</sup> (33.997)
8	2.4700 <sup>a</sup> (755.39)	2.5357 <sup>a</sup> (876.17)	0.1894 <sup>abc</sup> (0,5538)	2.0290 <sup>abc</sup> (9880.7)	3.1980 <sup>abc</sup> (10757)	0.4678 <sup>ab</sup> (3.5856)	1.7419 <sup>a</sup> (123.11)

Crude extract level	Juveniles in roots	Total nematodes in roots	Stem diameter	Juveniles in soil	Total nematodes in a pot	Reproductive Factor	Reproductive Potential
10	1.7959 <sup>ab</sup> (460.27)	1.8333 <sup>ab</sup> (519.21)	0.2115 <sup>ab</sup> (0,6320)	3.5130 <sup>a</sup> (23731)	3.4559 <sup>ab</sup> (24250)	0.8117 <sup>a</sup> (8.0833)	1.2377 <sup>ab</sup> (62.395)
Nemacur	0.0000 <sup>c</sup> (0.0000)	0.0000 <sup>c</sup> (0.0000)	0.1870 <sup>bc</sup> (0,5440)	0.0000 <sup>d</sup> (0.0000)	0.0000 <sup>d</sup> (00000)	0.0000 <sup>c</sup> (0.0000)	0.0000 <sup>c</sup> (0.0000)
F-value	4.31	3.69	2.80	3.56	6.66	4.12	3.52
LSD <sub>0.05</sub>	1.2392	1.3164	0,0351	2.0030	1.6089	0.3951	0.9250
P-value	0.0016**	0.0045**	0.0209*	0.0056**	0.0000**	0.0022**	0.0060**

<sup>a</sup>Column means followed by the same letter are not significantly different at  $P \leq 0.05$ , according to Fisher's least significant difference. Values in brackets are untransformed means. \*Significant ( $P \leq 0.05$ ); \*\*Highly Significant ( $P \leq 0.01$ )

Table 2. Effect of *Maerua angolensis* on plant growth variables and nematode eggs in roots

Cultivar	Number of Sprouts <sup>x</sup>	Plant Height	Shoot mass	Dry Shoot mass	Eggs in Roots
Mbonisweni	0,4758 <sup>a</sup> (2,1143)	1,8498 <sup>a</sup> (73.691)	1,3504 <sup>a</sup> (26,158)	0,8775 <sup>a</sup> (7,6054)	0,3828 <sup>b</sup> (34.286)
Mganduzweni	0,3502 <sup>b</sup> (1,2867)	1,6932 <sup>b</sup> (50.169)	0,9525 <sup>b</sup> (9,792)	0,5771 <sup>b</sup> (3,2423)	1,0371 <sup>a</sup> (85.675)
F-value	20.39	19.32	25.26	26.62	7.16
LSD <sub>0.05</sub>	0,0560	0,0717	0,1593	0,1172	0,1094
P-value	0.0000**	0.0001**	0.0000**	0.0000**	0.0103*

<sup>x</sup>Column means followed by the same letter are not significantly different at  $P \leq 0.05$ , according to Fisher's least significant difference. Values in brackets are untransformed means. \*Significant ( $P \leq 0.05$ ); \*\*Highly Significant ( $P \leq 0.01$ ).



Figure 1. Cassava cv. 'Mbonisweni' inoculated with *Meloidogyne incognita* and treated with *Maerua angolensis*

Even though there was evidence of nematode viability on indicator tomato plants, the current study was not conclusive. Higher nematode levels were observed in soils with plants exposed to plant extracts compared to the negative controls and the root system of cassava. The application of *M. angolensis* plant extracts seem to increase *M. incognita* populations in pots and reproductive factor of the nematodes. The observations contradict reports made on tomato plants by Khosa et al. (2021; 2020a; 2020b; 2013). Khosa et al. (2020a; 2020b) reported that *M. incognita* eggs and second-stage juveniles were highly sensitive to *M. angolensis* under *in vitro* and greenhouse conditions. Khosa et al. (2020b) using a CARD Model demonstrated that *M. angolensis* has very

high potency on hatching and mortality rate of *M. incognita*. Stem extracts of *M. angolensis* have also been reported to control *Haemonchus contortus* in sheep (Fouche et al., 2016). These variations could be because the powdered extracts largely depend on water to dissolve, it is possible that the outcomes could be different if the plant extracts used are applied in a liquid form.

Secondary metabolites in plants have been used as defensive strategies against insect pests and or pathogenic organisms (Sithole et al., 2021). Because of the growing interest to find an environment-friendly way of managing root-knot nematodes, different plant species have been reported to suppress nematode populations (Asif., 2017; Ntalli & Caboni, 2012). Some of the most investigated plants comprise elderberry (*Sambucus nigra* L.) (Akyazi, 2014) and garlic (*Allium sativum* L.), mugwort (*Artemisia vulgaris* L.) (Khosa et al., 2021).

Reproductive factor (RF) is used to describe host status, which is a measure of the nematode's ability to reproduce in a host. A reproductive factor greater than one, indicates that nematode reproduced in the plant whereas the RF less than one, indicates the inability of the nematode to reproduce in a plant. Sasser et al. (1984) included the galling index together with the RF to describe the plant status, when the gall index

is less than two and reproductive factor is less than one, the plant is said to be resistant. A plant is regarded as tolerant when the gall index is less than two and reproductive factor greater than one, whereas, when the gall index is greater than two and nematodes managed to reproduce, the plant is considered to be susceptible (Adegbite, 2017). In this study, RFs were greater than one except at 2 g, and there were few small galls on the plant roots and tubers, making the gall index in this case, less than two.

With the limited information gathered on the response of tolerant plants to plant extract used in nematode management, the results of the current study become difficult to explain. Dube (2016) observed a stimulative effect of cucurbitacin and cucurbitacin-containing plant extracts on *M. incognita* J2 hatch and mobility when exposed to low concentrations under *in vitro* conditions. The behaviour of plant-parasitic nematodes is continuously influenced by chemical cues in their environments, these chemicals can repel, attract or kill the nematode (McSorley, 2003). A larger body of evidence on the stimulatory effects of plant extracts has been associated mainly with beneficial nematodes, such as, *Sternernema* species (Madaure, Mashela & De Waele, 2017) and other plant beneficial organisms such as nitrogen-fixing bacteria (Mashela & Pofu, 2012). The mechanism by which plant extracts affect beneficial organisms has taken a variety of forms, from changing the soil properties to providing food (Widmer, Mitkowski & Abawi, 2002).

The effect on the cassava plant cannot be left out in the outcome of this study, especially considering that the negative control plants responded the same way as the plants exposed to extracts and positive control on most variables measured. Similar trends as in the study by Timana et al. (2021) were observed in the current one, where there was an increase of nematodes in soil than in roots and reproductive factor response, support this. The cassava plants' ability to tolerate the nematode could have had a much greater effect on nematode behaviour than plant extracts and knowing the chemistry of the plant extracts could help in better understanding this outcome.

A more comprehensive study needs to be conducted to substantiate the interactions

between, *M. incognita*, cassava plants, and *Maerua angolensis* plant extracts. This work could include the mode of interaction of the three, the effect of space, the active ingredients of the plant extract and time on the three above-mentioned factors.

## CONCLUSIONS

Based on RF values obtained in this study and the gall index of less than 2, both cultivars are tolerant to *M. incognita*. The cassava plants' ability to tolerate the nematode could have had a much greater effect on nematode behaviour than plant extracts and knowing the chemistry of the plant extracts could help in better understanding this outcome.

The study did not provide a conclusive relationship between the cassava plants, *M. angolensis*, and *M. incognita*. The use of *M. angolensis* plant extract did not bring any added benefit to the crop or suppress nematode populations in cassava cv. 'Mbonisweni' and cv. 'Mganduzweni'.

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