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Host status and sensitivity of cancer bush (*Sutherlandia frutescens*) to *Meloidogyne enterolobii*

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ABSTRACT

Cancer bush (*Sutherlandia frutescens*) is a medicinal plant with extensive medicinal properties and pharmacological applications. The attempts to increase the cultivation of the cancer bush are hampered by new incidences of nematode pests, such as *Meloidogyne enterolobii*. For an effective management strategy to be developed against a nematode pest, the host status and sensitivity of the test plant need to be empirically established. Hence, the objective of the study was to establish the host status and sensitivity of *S. frutescens* to *M. enterolobii*. To achieve this objective, *S. frutenscens* seedlings were subjected to 0, 25, 50, 125, 250, 625, 1250 and 3125 *M. enterolobii* eggs and second-stage juveniles (J2) under microplot and shade net conditions in 2024. At 56 days post-inoculation, plant and nematode variables were measured, and the reproductive factor (RF) was calculated. The RFs in both experiments were greater than 1 at nematode levels below 50, and below one at higher levels, establishing the *M. enterolobii* reproductive equilibrium position at 50 inoculum levels under these two growth conditions. However, not all cancer bush growth variables were affected by the *M. enterolobii* infection. The empirical evidence from this study indicates that the cancer bush is tolerant to *M. enterolobii.*

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Sutherlandia frutescens; root-knot nematodes; host status; *Meloidogyne enterolobii;* reproductive factor

Introduction

Cancer bush (*Sutherlandia frutescens* (L.R.) *Br.*) is an indigenous medicinal plant with a wide ecological diversity found in places like Namibia, Botswana and Zimbabwe (Masenya et al. 2020). The leguminous plant contains many essential bioactive chemicals with clinically verified pharmacological activities, such as cancer inhibitors, and also has pharmacological uses, such as boosting immunity in HIV patients (Shaik et al. 2011). Globally, large-scale cultivation is being adapted with current modern technologies to try to meet the need for plant-based medicinal products and ingredients for food (Makgato et al. 2020). South Africa's need for indigenous medicinal plants is projected above the current 20,000 tonnes per year, the international community's desire for alternative medical items is expected to follow suit as the population continues to grow (Noorhosseini et al. 2017; Asong et al. 2019; Nsibanyoni et al. 2023).

As a result of global warming, new plant-pest associations, with reduced life cycles per year and sex ratio changes, are on the rise, posing a growing concern for control of pests, such as nematodes (Nkosi 2019). Plant-parasitic nematodes (PPNs) are classified as agriculture's most damaging pests (Jones et al. 2013; Nkosi 2019), causing a drastic reduction in yield. *Meloidogyne enterolobii,* which has a 15-day life cycle (Collett et al. 2021), is becoming an increasingly major danger to most crops across the globe, with fewer solutions for managing nematodes. Currently, despite the existence of nematode resistance Mi genes in some crops, *M. enterolobii* is still among the most destructive and dominant root-knot nematode (RKN) species (Silva et al. 2017). In South Africa, the nematode has been detected in guava (*Psidium guajava*) and potato (*Solanum tuberosum*), and it has proven to be destructive in both crops (De Waele and Elsen 2007). Agricultural output losses amounting to billions of rands per year are blamed on plant parasitic nematodes, even though these organisms are tiny (usually about 1 mm in length). Kumar et al. (2020) shows that overall, plant-parasitic nematodes caused 21.3% crop losses amounting to Rs. 102,039.79 million (1.58 billion USD) annually; the losses in 19 horticultural crops were assessed at Rs.

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50,224.98 million, while for 11 field crops, it was estimated at Rs. 51,814.81 million. The *Meloidogyne graminicola*, commonly known as the rice root-knot nematode, resulted in economic yield losses of Rs. 23,272.32 million in rice (*Oryza sativa*). Citrus (Rs. 9828.22 million), banana (*Musa acuminata*) (Rs. 9710.46 million) among fruit crops and tomato (*Solanum lycopersicum*) (Rs. 6035.2 million), brinjal (*Solanum melongena L.*) (Rs. 3499.12 million) and okra (*Abelmoschus esculents L.*) (2480.86 million) among the vegetable crops suffered comparatively more losses in India. *Meloidogyne javanica* is the first root-knot nematode species to be detected on *S. frutescens,* and it is said to reproduce and affect the growth and yield of *S. frutescens* (Raselabe 2017). There is a need for evaluation of more PPNs, such as *M. enterolobii,* in the production of cancer bush, to establish whether the RKNs will reproduce and reduce the growth of the plant. This will allow the use of the crop as an alternative strategy for management of the selected RKN species in rotational systems or to decide what management strategies can be deployed to control the selected RKNs in the test crop (Talwana et al. 2016). The objective of this study was to determine whether *M. enterolobii* would reproduce on *S. frutescens* and reduce plant growth.

Materials and methods

Description of the study area

Two separate experiments were carried out at the University of Mpumalangas (25.4371°S, 30.9818°E) Mbombela campus farm under microplot-field and shade-net conditions from May to August 2024, with temperature ranging between 26 °C and 33 °C, with an average of 162 mm precipitation per annum.

Preparation and collection of plant material and inoculum

Cancer bush seeds were purchased from the Mountain Herb Estate Nursery in Gauteng province, South Africa. For both experiments, 20-cm-diameter plastic pots were filled with 27000 ml steam pasteurised (300 °C for one hour) sand and loam soil at a 3:1 (v/v) ratio and then placed under a microplot field, an open field with pots directly dug into the field and shade-net conditions, structured with a green net-covered roof and pots placed on the cement floor, using a randomised complete block design (RCBD). Under the microplot conditions, blocking was necessary against the shade and under shade nets, blocking was against the uneven coverage of the nets on the side of the nets where wind was causing variation in plant development. Pots were spaced at 0.5 × 0.5 m inter-row and intra-row spacing in both planting systems, whereas under microplot conditions, pots were inserted at 30 cm depth.

Scarification of the seeds was done in hot water (80 °C) overnight to enhance germination before planting. Five (5) seeds were directly planted per pot, and 2 weeks after germination, the cancer-bush seedlings were then thinned to one seedling per pot. Seedlings were hardened for seven days through interment withdrawal of water before transplanting, seven days after transplanting, eight treatments of 0, 25, 50, 125, 250, 625, 1250 and 3125 *M. enterolobii* eggs and second-stage juveniles (J2) were applied to respective seedlings, with each replicated seven times. Roots of nematode-susceptible kenaf (*Hibiscus cannabinus* L.) grown in a greenhouse were harvested for J2 and eggs of *M. enterolobii* in preparation for the inoculum. The inoculum was applied on the cardinal points of the plant with 3-cm-deep holes around the test plant. Plants were irrigated with 250 ml of tap water when the soil moisture level was below 60%. Daily inspections for pests and diseases were conducted throughout the duration of the study. Pest' management was done when needed.

Data collection

The height of the plants was measured using a measuring tape to the nearest millimetre 56 days after plants were inoculated. The number of branches per plant was counted before cutting it at the soil line for drying. The dry shoot mass was determined by placing the samples in an oven set at a temperature of 52 °C for 72 hours (Makhado 2020). The stem diameter was measured using a Vernier calliper at 5 cm above the cut stem end. Root systems were removed from the pots, washed under running tap water to remove any residual soil. Subsequently, the roots were dried using a laboratory paper towel to eliminate any excess water (Makhado 2020). Root galls were assessed using a 5-point scale system, where 0 represented no galls, 1 represented 0.5 galls, 2 represented 3–10 galls, 4 represented 31–100 galls and 5 represented more than 100 galls (Taylor and Sasser 1978). Before assessing root gall, the fresh root mass was measured.

The maceration and blending method (Hussey and Baker 1973) was employed to extract nematodes from root material. The infected roots were cut into 1 cm pieces and placed for 90 s in a solution of 1% sodium hypochlorite (NaOCl) before blending for 30 s. The mixture was then passed through a series of sieves with pore diameters of 125, 75 and 25 µm, with pressured flow of tap water used to assist in the filtration.

Nematodes obtained from the 25 µm sieve were quantified utilising a light microscope and subsequently analysed for morphological identification.

Nematode extraction from soil samples was achieved using the modified sugar flotation and centrifugation method (Marais et al. 2017). Briefly, soil from each pot was thoroughly mixed, and a 500 ml sample was placed in a bucket containing five litres of tap water and stirred to suspend the nematodes. When the swirl had stopped, an aliquot was quickly passed through a 125, 75 and 25 µm nest of sieves. Nematodes on the bottom sieve were washed into 100 ml centrifuge tubes. A teaspoon of kaolin was then added to each tube and centrifuged at 2000 rpm for 5 min. The top liquid aliquot was discarded, the tubes were then topped with a sugar solution of 624 g sugar/L and stirred to bring the solutes into suspension before centrifuging the mixture for 1 min at 2000 rpm to suspend nematodes in the filtrate. The aliquot was then passed through 25 µm mesh sieves. The sugar was rinsed off by placing the sieve containing nematodes under running tap water, and the nematodes collected were poured into a 100 ml container for counting under a stereomicroscope at a magnification of 40×.

Data analysis

The plant growth variables and nematode data were subjected to analyses of variance (ANOVA) through Statistix 10 software. A Shapiro–Wilk normality test was used to test for deviation from normality in each standardised residual variable (Gomez 1984). To minimise variation amongst variables, the data were transformed using the $\log_{10}(x+1)$ transformation (Mbatyoti 2018). Fisher's Least Significant Difference Test ($P \leq 0.05$) was used to achieve the mean separation.

Results and discussion

In both the microplot and shade net experiments, plant growth variables, namely number of branches, chlorophyll, stem diameter, plant height, fresh shoot mass and dry shoot mass, were not significantly ($P > 0.05$) affected by the treatments. Under Microplot conditions, all the nematode variables, namely root galls, J2 in roots, eggs in roots, final population (PF), and reproductive factor (RF), were significant ($P \leq 0.05$) except J2 in soil. The significant nematode variables had a total treatment variation (TTV) of 82%, 70%, 64%, 94%, and 91%, respectively. Under shade net conditions, three of the nematode variables, namely root galls, J2 in soil and RF, were not significant. The J2 in roots, eggs in roots and PF were significant with TTVs of 71%, 78% and 76%, respectively. Seinhorst (1967) explains nematode resistance in terms of two fundamental concepts, which are host-status and host-sensitivity. The host-status and host-sensitivity ideas are the starting point for the identification of plant nematode resistance status (Seinhorst 1967). According to Ngobeni et al. (2012), the reproductive factor measures the nematode's capacity for reproduction on a particular host, and it is used to characterise host status. The RF helps evaluate whether a plant is a host or non-host to a given nematode (Seinhorst 1967). The RF values less than one indicate that the test nematode was unable to feed and reproduce on the test plants. In contrast, values larger than one indicate that the nematodes effectively established feeding sites and reproduced on the test plants (Makhado 2020). In the present study, at the nematode inocula levels ≤50, the RF was greater than 1, whereas at inocula levels ≥125, the RF was less than 1 under microplot conditions (Table 1). Under shade net conditions, the RF was greater than 1 at inocula levels ≤25 and less than one at inocula levels ≥125 (Table 1). This

Table 1. Response of root galls, Meloidogyne enterolobii second-stage juveniles (J2) in soil, J2 in roots, eggs in roots, final nematode population density (PF) and reproductive factor (RF) on cancer bush under 2 different conditions.

Treatment	Root galls	J2 in roots	Eggs in roots	PF	RF	J2 in soil	Eggs in roots	PF	RF
			Microplot Experiment				Shade Net Experiment		
25	0.38^{a}	2.45^{ab}	2.35^{b}	2.84^{b}	1.46^{a}	0.28^{c}	1.85^{a}	2.35^{b}	1.01^{a}
	(1.57)	(300)	(257.14)	(714.29)	(28.57)	(14.29)	(142.86)	(285.71)	(11.43)
50	0.35^{a}	2.47^{ab}	2.48^{ab}	2.95^{ab}	1.28^{b}	1.23^{b}	0.86^{b}	1.76^{c}	0.60^{b}
	(1.29)	(357.14)	(385.71)	(971.43)	(19.43)	(85.71)	(42.86)	(214.29)	(4.29)
125	0.48^{a}	2.15^{ab}	2.36^{b}	2.87^{b}	0.84^{c}	1.80^{ab}	1.80^{a}	2.50^{ab}	0.55^{b}
	(2.14)	(285.71)	(271.43)	(785.71)	(6.29)	(114.29)	(114.29)	(328.57)	(2.63)
250	0.44^{a}	2.42^{ab}	2.46^{ab}	2.88^{b}	0.61^{d}	1.47^{ab}	2.11^{a}	2.48^{ab}	0.35^{c}
	(1.86)	(300)	(371.43)	(814.29)	(3.26)	(85.71)	(142.86)	(314.29)	(1.26)
625	0.42^{a}	2.44^{ab}	2.59^{ab}	2.94^{ab}	0.38^{e}	1.85^{ab}	2.09^{a}	2.54^{ab}	0.19^{cd}
	(1.71)	(300)	(414.29)	(885.71)	(1.42)	(128.57)	(128.57)	(357.14)	(0.57)
1250	0.44^{a}	2.11^{b}	2.65^{a}	3.00^{ab}	0.26^{e}	1.31^{b}	2.29^{a}	2.60^{ab}	0.12^{de}
	(1.86)	(257.14)	(457.14)	(1100)	(0.90)	(114.29)	(214.29)	(442.86)	(0.35)
3125	0.35^{a}	2.61^{a}	2.61^{ab}	3.04^{a}	0.13^{f}	2.38^{a}	2.09^{a}	2.84^{a}	0.09^{de}
	(1.43)	(428.57)	(457.14)	(1142.9)	(0.37)	(257.14)	(271.43)	(714.29)	(0.23)
$LSD_{0.05}$	0.14	0.47	0.27	0.16	0.12	0.93	0.72	0.48	0.19

yColumn means ± standard error, followed by the same letter, were not different ($P \leq 0.\ 05$) according to Fisher's Least Significant Difference test.

is an indication of the nematode population level reaching the reproductive equilibrium level. Similar results were observed by Timana (2023), where the RF rates were highest at certain levels of inoculum and then decreased with an increase in inoculum, when testing the host response of cassava cv. 'Mbonisweni' to *Meloidogyne incognita*. The expected trend in susceptible plants is that the final nematode population (PF) density increases as the initial population (Pi) increases (Pofu et al. 2017; Timana 2023). It is believed that the factors such as competition for infection sites in roots and food scarcity cause a decline in PF and stabilises around the equilibrium density at which the plant can supply enough food to maintain the population density on the host (Timana 2023).

In the present study, there were differences in the appearance of root galls in the experiments. The root galls had a significant impact on the treatments under the microplot experiment, while there were no root galls visible under the shade net experiment (Table 2). According to Eisenback and Triantaphyllou (2020), the absence of the root galls does not mean there are no nematodes present, which is supported by Ansari et al. (2019) where 10 medicinal plants were tested as hosts and chamomile (*Matricaria chamomilla* L.) had fewer galls but was still hyper susceptible to *Meloidogyne javanica*. Multiple root activities, including water intake, are disrupted when galls develop (Engelbrecht et al. 2021). Vilela et al. (2023) disorganise the vascular system, influencing the formation of cultures. The observation of root galls formation in the current study under microplot conditions might be due to the climatic stress the plants underwent, making them susceptible to gall formation by nematodes, whereas under shade net conditions, the environment is more controlled and offers less stress to the plants. Numerous plant species possess nematicidal constituents that suppress nematode populations and improve plant health (Azeem et al. 2025). Medicinal plants such as cancer bush contain secondary metabolites, such as phenolic compounds, steroids, triterpenes, anthraquinones, flavonoid glycosides, saponin glycosides, condensed tannins and hydrolysable tannins they use to kill or poison the threatening pests (Van Wyk and Prinsloo 2020). Azeem et al. (2025) observed that *Moringa Oliefera* both alone and combined with rhizobacterial strains effectively reduce *M. incognita* population. *M. Oliefera* contains similar components to *S. frutescens* as they are both medicinal plants. *Sutherlandia frutescens* under shade net conditions might have used the secondary metabolites as a resistance mechanism against the *M. enterolobii,* hence the absence of the galls. However, the phenomenon of the influence of the

Table 2. Partitioning mean sum of squares of root galls, second-stage juveniles (J2) in soil, J2 in roots, eggs in roots, final population (PF) and reproductive factor (RF) of Meloidogyne enterolobii on cancer bush under 2 different conditions.

		Root galls		J2 in soil		J2 in roots		Eggs in roots		PF		RF	
Source	DF	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)
Microplot Experiment													
Replication	6	0.02194	10.94	0.74620	13.38	0.63548	10.61	0.03904	0.69	0.01624	0.21	0.01086	0.54
Treatment	7	0.16267	81.10**	4.13017	74.07^{ns}	5.14823	85.99**	5.56093	98.19**	7.56077	99.50**	1.99455	98.85**
Error	42	0.01597	7.96	0.69984	12.55	0.20333	3.40	0.06362	1.12	0.02184	0.29	0.01229	0.61
Total	55	0.20058	100	5.57621	100	5.98704	100	5.66359	100	7.59885	100	2.01770	100
Shade Net Experiment													
Replication	6	0.00	0.00	0.68067	11.57	0.79737	17.44	0.44837	8.44	0.28308	4.45	0.04685	5.35
Treatment	7	0.00	0.00	4.46724	75.92**	2.87042	62.80^{ns}	4.42023	83.25**	5.87915	92.43**	0.79662	90.98**
Error	42	0.00	0.00	0.73589	12.51	0.90329	19.76	0.44096	8.31	0.19836	3.12	0.03211	3.67
Total	55	0.00	0.00	5.88380	100	4.57108	100	5.30956	100	6.36059	100	0.87558	100

**Significant $P \leq 0.05$, ^{ns}Not significant $P > 0.05$.

environment on the levels of secondary metabolites still needs to be investigated fully, as it is unclear. Galling was also one of the symptoms observed by Raselabe (2017) when screening nematodes on the roots of *S. frutescens* in a field trial. The findings were similar to those made by Ansari et al. (2019), where the medicinal plants sorrel (*Rumex acetosella L.*) and horehound (*Marrubium vulgare L.*) had a significantly higher number of galls. Conversely, this study had a root galling index (GI) that is less than 2 in all the nematode inocula levels under the microplot.

Host status and host sensitivity are shown by tolerance, sensitivity and resistance. In the study, the host status is positive at lower levels, indicating that *S. frutescens* is a host to *M. enterolobii* as the nematodes were able to reproduce. Raselabe (2017) discovered that *S. frutescens* is susceptible to different root-knot nematodes (RKN) that were found in the soil, meaning that the plant allowed nematode reproduction and suffered negative growth, which led to yield losses. The root system of *S. frutescens* in all the experiments appeared to be very small, which could have had an impact on the decrease of the RF at higher inoculation levels. The feeding sites might have been too populated and not been able to provide food for the large nematode populations, hence the reduction in the PF. Some medical plants are susceptible, whereas some are resistant to RKNs. Mendonça et al. (2017) investigated how seven different species of medicinal plants responded to *Meloidogyne paranaensis*, assessing the results using the reproduction factor and gall index. *Melissa officinalis*, *Hypericum perforatum* (eola-weed) and *Pfaffia glomerata* (Brazilian ginseng) were all highly susceptible to *M. paranaensis*. *Pogostemon cablin* (patchouly) was categorised as susceptible due to its intermediate response, whereas *Cordia verbenacea* was categorised as resistant, with *Artemisia annua* (sweet sagewort) and *Catharanthus roseus* (Madagascar periwinkle) being extremely resistant. *Catharanthus roseus* stood out due to its high gall index, which prevented the nematode from reproducing.

Conclusion

Sutherlandia frutescens was tolerant to *Meloidogyne enterolobii* as the gall index (GI) was less than 2 under microplot conditions and the reproductive factor (RF) was less than 1, particularly at high inocula levels, while growth was not reduced relative to the control. *Sutherlandia frutescens* can be cultivated and produced on soils that are infested by *M. enterolobii.* The study also reveals that microplot conditions favour *M. enterolobii* reproduction better than shade net conditions. Therefore, it might serve as a valuable component in nematode management strategies, as a rotation crop.

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