

## PROTECTION OF BAMBARA GROUNDNUTS (*VIGNA SUBTERRANEA*) FROM ROOT-KNOT NEMATODES: A CLIMATE SMART APPROACH

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### Abstract

Bambara groundnut (BG) is third most important legume in sub-Saharan Africa. Currently, there are no commercial varieties, and reports of *Meloidogyne* species impacts have been made without empirical evidence. The withdrawal of synthetic nematicides has increased the demand for alternative products. Therefore, the objectives of this study were to determine the resistance status of BG varieties to *M. incognita* and potential use of *Maerua angolensis* extract in the control of *M. incognita* and growth of BG varieties. Screening experiment: six BG varieties were inoculated with  $\approx 5000$  *M. incognita* eggs and second-stage juveniles under greenhouse condition. Management experiment: 6 x 5 factorial experiment under similar conditions. The first factor consisted of retained BG varieties and the second factor was made up of *M. angolensis* extract levels, fenamiphos, and untreated control. At 130 days after inoculation, all the six varieties were hosts to *M. incognita* at varying degrees, but plant growth variables were not sensitive to the nematode. Plant extracts had no direct effects on nematode numbers but increased the BG varieties tolerance to nematode attack

**Key words:** Bambara groundnut, host sensitivity, host status, *Maerua angolensis*, *Nemacur*.

### INTRODUCTION

Bambara groundnut (*Vigna subterranean*) is the third most important legume crop after groundnut (*Arachis hypogea*) and cowpea (*Vigna unguiculata*) in Sub-Saharan Africa (Hillocks et al., 2012). Bambara groundnut yields in Africa are comparatively small, ranging between 650 and 850 kg/ha (Jada et al., 2011). Apart from genetic potential, diseases and pests such as nematodes are one of the key causes of low Bambara groundnut yields (Kankam and Adomako, 2014).

As a result, pest and disease management is an important criterion for increasing agricultural productivity of the crop (Dias et al., 2015). Although evidence suggests that the crop is not free of pests and diseases, as previously documented by Murevanhema and Jideani (2013), very little research has been undertaken on pests and diseases of Bambara groundnut. This has a negative impact on the crop's production. *Maerua angolensis* has been found to be effective against nematodes (Khosa et al., 2020). Investigating effective control techniques to reduce yield losses observed in small-scale farmer production systems is

critical. Although chemical management with nematicides has proven to be the most effective, nematicides are often expensive and out of reach for small-scale farmers. Due to their harmful impact on the environment, non-target organism many nematicides have been taken off the agrochemical market. It is becoming more popular to identify and use local plant resources for nematode control (Khosa et al., 2020). Due to the growing problem of environmental pollution caused by the use of persistent pesticides, this strategy of managing *Meloidogyne* species is appealing (Kankam and Adomako, 2014.).

Several studies have demonstrated that plant extracts from several indigenous plants especially neem (*Azadirachta indica*) products are efficient against insects and nematodes (Khosa, 2021; Khosa, 2020; Mashela et al., 2017). *Maerua angolensis* plant extracts have been extensively reported to be effective in the management of *Meloidogyne incognita* on tomato plants (Khosa et al., 2021) but their effects on nematodes attacking traditional crops such as Bambara groundnuts has not been empirically studied. Hence, two studies were

carried out to determine the host status and host sensitivity of Bambara groundnuts and to test the efficacy of *Maerua angolensis* in the treatment of *Meloidogyne incognita* on the same crop.

## MATERIALS AND METHODS

The research was carried out under greenhouse conditions at the University of Mpumalanga, (25.4365° S, 30.9818° E), Nelspruit, South Africa. Greenhouse temperatures were set between 25 and 30°C, with thermostatically activated fans and wet-wall at opposite ends.

Screening trial: Thirty-centimetre diameter plastic pots were placed on greenhouse benches at the spacing of 0.3 m intra- and inter-row spacing. Six Bambara groundnut varieties ('DP-W', 'BR-W', 'BS-W', 'CW-W', 'BB-W', and 'DB-W'), were then planted in separate pots. Four seeds of each variety were separately planted in each pot. At two true-leaf-stage, the seedlings were thinned to one plant per pot. A week after thinning, Bambara groundnut seedlings were inoculated with 5000±20 *M. incognita* eggs and second-stage juvenile (J2) using a 20 ml plastic syringe by putting them on cardinal stem points in a 2½ cm deep hole. A randomized complete block design (RCBD) with ten replicates was used to arrange the treatments.

Management trial: The location and experimental setup were as described previously in screening trial, except that treatments consisted of *M. angolensis* extract leaf powder levels applied on different varieties. A week after inoculation of seedlings with nematodes, treatments of medicinal plant meal extracts were spread thinly by hand around the base of seedlings on the soil surface and then lightly mixed with topsoil. The experiment constituted of a 6 x 5 factorial arrangement, the first factor being six Bambara groundnut varieties and the second factor consisted of three *M. angolensis* leaf powder levels; (5, 10 and 15 g/pot that translated to 697, 1394, and 2091 kg/ha, respectively) plus a standard synthetic, commercial nematicide, Nemacur® 10GR (a.i. fenamiphos) at a rate of 697 kg/ha, as well as a negative control (plants inoculated with nematode without any

treatment). The treatments were also arranged in an RCBD, with five replications.

## DATA COLLECTION

Plant variables: At 56 days after inoculation, plant height was measured from the crown to the end of the flag leaf, stems were cut at the soil line, and stem diameter estimated at 3 cm above the cut end using a manual vernier caliper (Model no: 464-9952, RS PRO, South Africa). The chlorophyll content was measured using the chlorophyll meter (Minolta spad-502, Hangzhou, China). Shoots were weighed and then dried in an oven for 72 hours at 70 °C. The root systems were taken up from the soil, washed to eliminate any soil particles, blotted dry, and weighed.

Nematode variables: The nematodes were extracted from the entire root system using the maceration and blending process (Fourie et al., 2017). The soil in each pot was well mixed, and 250 mL of soil samples were collected, with nematodes extracted from it using a sugar floatation and centrifugation process (Fourie et al., 2017). Eggs and J2 from root and J2 from the soil were counted under a stereomicroscope (Olympus Corporation Tokyo 163- 0914, CX23RTFS2) from a 1 ml aliquot of each sample. The final nematode population density (Pf) was calculated by adding total eggs and J2 from the root system to total J2 from the soil. Reproductive potential (RP = total eggs and J2 in root/fresh root mass) and Reproductive factor (RF = final nematode population (Pf) divided by the initial nematode inoculated (Pi)) indicators of nematode's ability to reproduce were computed (Kayani and Mukhtar, 2018).

## DATA ANALYSIS

Plant growth and nematode data were subjected to analysis of variance (ANOVA) using Statistix 10 software. Prior to ANOVA, data were subjected to Shapiro-Wilk normality test for homogeneity determination. Non-continuous data were transformed using  $\log_{10}(x+1)$  to homogeneous variance. Means of significantly different variables were separated using the Fishers' least significant difference (LSD) test at 0.05 level.

## RESULTS AND DISCUSSIONS

For the screening experiment: There were no significant differences ( $P > 0.05$ ) in all measured nematode variables and plant growth variables among varieties, except for plant runner length which was highly significant ( $P \leq 0.01$ ). The reproductive potential and reproductive factors were also not significantly different among cultivars, while there were all greater than one. Variety 'CW-W' had the longest runners compared to all other varieties, which were not different from each other (Figure 1).

For the management experiment: the first-order interaction between *Maerua angolensis* plant extracts and Bambara groundnut landraces were highly significant ( $P \leq 0.01$ ) for all nematode variables measured (Table 1). There were no different in the effectiveness of the

plant extract levels on nematodes population densities. Plant extract levels reduced total nematodes in roots. However, plants treated with Nematicur® had lower total nematodes in roots compared to plant treated with plant extract. Nematicur® significantly reduced all nematode variables in all Bambara groundnut landraces compared to plants exposed to plant extracts, except landrace 'DB-W' where Nematicur® effects on the total nematodes were not significantly different to those of plant extracts. Nematodes reproduced and increased in numbers in all plants treated with all the different plant extracts level, however, Nematicur reduced the total nematodes in all varieties except var. 'DP-W', 'CW-W' and 'DB-W' were nematodes increased in numbers. Plant extracts had no direct effects on nematode numbers but increased the Bambara groundnut varieties tolerance to nematode attack.

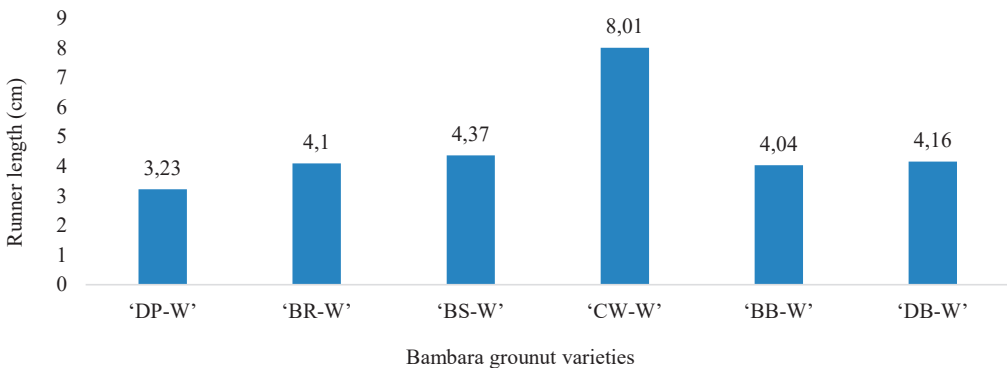


Figure 1: Effects of Bambara groundnut varieties on runner length

Table 1. Interactive effects of Bambara ground variety and *Maerua angolensis* plant extracts on *Meloidogyne incognita* variables

Variety	Plant extract															
	Total nematode in roots					Juveniles in soil					Total nematode					
	0 <sup>x</sup>	5	10	15		Nemacur ®	0	5	10	15		Nemacur ®	0	5	10	15
'DP-W'	3.27 <sup>cde</sup> f (1950)	3.39 <sup>abcde</sup> f (4770)	3.50 <sup>abcde</sup> f (5610)	3.25 <sup>cdefg</sup> (2400)	2.22 <sup>ghi</sup> (600)	4.24 <sup>ab</sup> (175000)	4.50 <sup>a</sup> (32000)	4.30 <sup>a</sup> (26000)	4.61 <sup>a</sup> (50000)	3.08 <sup>cd</sup> (6000)	4.20 <sup>abcd</sup> (19450)	4.63 <sup>ab</sup> (43770)	4.48 <sup>abc</sup> (34610)	4.70 <sup>a</sup> (57400)	4.03 <sup>bcd</sup> (11600)	
'BR-W'	4.00 <sup>b</sup> (1545 0)	3.26 <sup>cdef</sup> f (2160)	3.33 <sup>abcde</sup> f (4470)	3.32 <sup>bcdef</sup> (4320)	1.92 <sup>ij</sup> (225)	4.27 <sup>a</sup> (23000)	4.18 <sup>ab</sup> (19000)	4.49 <sup>a</sup> (38000)	4.56 <sup>a</sup> (38000)	0.00 <sup>e</sup> (000)	4.66 <sup>a</sup> (49450)	4.32 <sup>abcd</sup> (25160)	4.56 <sup>ab</sup> (46470)	4.73 <sup>a</sup> (54320)	1.92 <sup>f</sup> (225)	
'BS-W'	3.97 <sup>ab</sup> (1395 0)	3.72 <sup>abcde</sup> (9000)	3.74 <sup>abcd</sup> (7890)	3.31 <sup>bcdef</sup> (2520)	1.54 <sup>i</sup> (75)	4.55 <sup>a</sup> (38000)	4.42 <sup>a</sup> (40000)	4.62 <sup>a</sup> (59000)	4.68 <sup>a</sup> (50000)	2.22 <sup>d</sup> (3000)	4.71 <sup>a</sup> (51950)	4.57 <sup>ab</sup> (55000)	4.79 <sup>a</sup> (73890)	4.70 <sup>a</sup> (52520)	2.54 <sup>e</sup> (3075)	
'CW-W'	2.59 <sup>gh</sup> (450)	3.33 <sup>abcde</sup> f (2880)	3.52 <sup>abcde</sup> (4260)	3.43 <sup>abcde</sup> f (3330)	2.88 <sup>efgh</sup> (750)	4.62 <sup>a</sup> (46000)	4.40 <sup>a</sup> (28000)	4.31 <sup>a</sup> (23000)	4.54 <sup>a</sup> (41000)	3.24 <sup>bc</sup> (16000)	4.76 <sup>a</sup> (59450)	4.51 <sup>abc</sup> (33880)	4.47 <sup>abc</sup> (33260)	4.64 <sup>ab</sup> (51330)	3.84 <sup>d</sup> (16750)	
'BB-W'	3.86 <sup>abc</sup> (8775)	3.49 <sup>abcde</sup> f (4680)	3.57 <sup>abcd</sup> (9240)	3.16 <sup>defg</sup> (2100)	1.54 <sup>i</sup> (75)	4.19 <sup>ab</sup> (16000)	4.49 <sup>a</sup> (34000)	4.64 <sup>a</sup> (56000)	4.37 <sup>a</sup> (26000)	2.22 <sup>d</sup> (3000)	4.55 <sup>ab</sup> (35775)	4.65 <sup>a</sup> (46680)	4.78 <sup>a</sup> (72240)	4.57 <sup>ab</sup> (39100)	3.07 <sup>e</sup> (3075)	
'DB-W'	2.86 <sup>efg</sup> (750)	3.38 <sup>abcde</sup> f (2490)	3.26 <sup>cdef</sup> (3150)	3.64 <sup>abcd</sup> (6420)	3.35 <sup>abcdef</sup> (3375)	4.24 <sup>ab</sup> (18000)	4.54 <sup>a</sup> (45000)	4.44 <sup>a</sup> (43000)	4.49 <sup>a</sup> (32000)	3.24 <sup>bc</sup> (10000)	4.25 <sup>abcd</sup> (18750)	4.63 <sup>ab</sup> (57490)	4.57 <sup>ab</sup> (55150)	4.61 <sup>ab</sup> (41420)	3.89 <sup>cd</sup> (16375)	
F-value			3.70					2.40							3.66	
LSD <sub>0.05</sub>			0.6642					1.0103							0.6158	
P-value			0.0000					0.0019							0.0000	

<sup>x</sup>Column means followed by the same letters are not significant different at P ≤ 0.05 according to Fisher's Least Significant Difference.

<sup>y</sup>X values in brackets are untransformed means [log<sub>10</sub>(X+1)].

The host status of a plant to nematodes is usually determined by any one or both of the following indicators, reproductive potential and reproductive factor (Anwar and McKenry, 2010). The reproductive factor of the nematode, quantifies the nematode reproduction through a ratio of initial versus final nematode populations on the plant, making this a perfect indicator under a closed environment where soil nematodes can be manipulated (Kayani and Mukhtar, 2018). When the reproductive factor on a particular host is less than one, it indicates that the nematode cannot reproduce on that host hence that plant is not a host (Kayani and Mukhtar, 2018). If the reproductive factor is greater than one, the nematode has multiplied successfully on the host (Pofu et al., 2010). The reproductive factor is a basic indicator of nematode reproductive potential and hence gives a good indication of a plant's level of resistance (Steyn et al., 2012). On the other hand, the reproductive potential indicator uses the ratio of nematodes in roots as a function of the total fresh root mass (Anwar and McKenry, 2010). The reproductive potential of a nematode can be used as an indicator of whether a plant is susceptible, tolerant, or resistant to the presence of nematode. Susceptible plants are those that will be affected by the presence of nematodes and result in plant growth failure, while a tolerant plant is able to withstand the presence of nematode and continue to grow without being affected (Jones et al., 2013). Resistant plants restrict the presence of nematode at all, if nematodes are exposed to such plants they will not feed at all (Ralmi et al., 2016). According to Pofu et al. (2020) judgement of host status indication, all the Bambara groundnut varieties in the current study were a host of *M. incognita*. *Meloidogyne incognita* was able to penetrate and multiply in all six varieties (Steyn et al., 2012). Muhamman et al. (2010) reported Bambara groundnut to be good host for *Meloidogyne javanica*, another *Meloidogyne* species. The current study hence expands on the host spectrum of Bambara groundnuts to nematode species. Varieties having lower reproductive factors will be appropriate for the management of root-knot nematodes (Mukhtar and Hussain, 2019).

On host sensitivity, the current study reports that nematodes had no effect on the plant growth variables between the inoculated and un-inoculated plants of the same variety, and varieties also had similar growth trends except for var. 'CW-W' which had longer runner lengths. The similarity in the growth variables of the varieties when exposed to nematodes and when nematodes are absent is an indication of low sensitivity (Pofu et al., 2010). *Meloidogyne incognita* is a major problem on Bambara groundnut, according to Kwerepe and Labuschagne (2004), who screened 15 Bambara groundnut landraces from Botswana and South Africa and found signs of resistance and tolerance to this nematode. The performance of South African varieties performance in the presence of nematodes contradicts reports made by Imegwu et al. (2014) and Ogbuji (1979), who observed stunted growth in Bambara groundnut types from Nigeria inoculated with a local population of *M. incognita* and *M. javanica*. Tobih et al. (2011) reported runner length as a good sign of a healthy plant able to utilize the soil nutrients optimally. Previous studies by McDonald and De Waele (1989) suggested that *M. javanica*-tolerant Bambara groundnut variety exists. Bambara groundnut length runner is important in determining the yield of the crop because it is where the pods are formed even though there is no documented literature on the relationship between the two. Mukhtar et al. (2014) and Mukhtar et al. (2013) reported that variation in plant response can be due to the genetic make-up of the host plant and different environmental factors. Karikari (2000) reported that varieties with a growth behavior like var. 'CW-W' have the potential for strategic usage as an intercrop, where it can provide faster ground cover and help restrict weed growth by spreading out. Variety 'CW-W' is known by the fact that it produces more flowers and matures earlier than other types (Berchie et al., 2010). In Bambara groundnuts, it is believed that the number of runner lengths is a good indicator of high productivity since flowers and pods are formed in the runner length. There is currently a scarcity of knowledge on the relationship between floral characteristics and pod production in Bambara groundnut. This information is critical for

understanding the pod formation ability of the Bambara groundnut, as well as designing the optimum selection method(s) for increasing pod production in the crop.

Following the environmental concerns produced by chemical control measures, the use of diverse sections of indigenous plants as botanical extracts has been increasingly essential in pest management in recent years (Mamun and Ahmed, 2011). It is apparent from the result of this study that plants with plant extract levels showed the best plant growth variables (stem diameter, runner length, fresh root mass, dry shoot mass, and high chlorophyll content) and development compared to plants treated with Nematicur®. According to Khosa et al. (2020), a higher shoot weight caused by the presence of *Marua angolensis* was observed in the years 2008 and 2011.

The lower rate of nematode multiplication in Nematicur® treated plants could be due to a lower rate of juvenile penetration into varieties, which would restrict nematode multiplication and result in a low fecundity rate (Mukhtar et al., 2013). Mnyambo et al. (2021) reported Nematicur® consistently reducing nematode densities in roots of all six cultivars. The current results contradict work by Khosa et al. (2020) who reported *M. angolensis* suppressing the nematode population densities significantly by 87 to 88%. Khosa et al. (2021, 2020), observed suppression of *M. incognita* caused by *M. angolensis* similar to the suppression caused by Nematicur® in 2008 and 2009. Moreover, *M. angolensis* did not suppress nematode densities in 2011 (Khosa et al., 2020), which supports the current study. Khosa et al. (2020) reported that the increase in plant growth variables caused by the presence of *M. angolensis* can result in more roots for the nematodes to reproduce in numbers.

The continuing growth of the plant without being compromising the presence of nematode is a sign that Bambara groundnut is tolerant to *M. incognita*. The influence of plant extracts results in improved root health, active root nodules for active nitrogen fixation, enhanced root anchorage, and good uptake of nutrients and water from the soil by the plant.

The improvement of plant growth variables of plants treated with *M. angolensis* and

*T. elegans* have been explained mostly using three phenomenon, firstly that the ability of plant extract to suppress nematodes results in plants growing in an environment of pest free hence better performance (Ugwuoke, Ukwueze and Ogwulumba, 2011). The second phenomenon, is that addition of plant extracts improves the soil conditions around the plants' rhizosphere (Khan et al., 2019). The improvement of soil conditions has received extensive attention in agronomic studies of organic amendments and disease suppression (Khan et al., 2019). The third concepts which is quickly gaining attention is the induction of resistance and/or defense reactions by compounds produced by plant extracts in treated plants (Thakur and Sohal, 2013). The concept of induced resistance is well studied in plant fungal and bacterial diseases, with elicitors being identified as inducers of such resistance (Thakur and Sohal, 2013). Induced plant resistance has been defined as the enhancement of a plant's defense against pests through extrinsic physical or chemical stimuli and these stimuli could be a pathogen, insect herbivore or wounding, beneficial microbe, or chemical agent (De Kesel et al., 2021). A further study could need to be conducted to comprehensively explain the relationship between Bambara groundnut varieties and *M. angolensis*.

The improved plant growth observed in plants treated with plant extract could be due to that more nutrients were readily available to the plants, which no doubt promoted vigorous growth to counteract the nematode's attack (Nworgu, 2006). The study is supported by the work of Khosa et al. (2020) who observed an increase of plant growth variables in plants treated with *M. angolensis*. According to the current findings, *Maerua angolensis* plant extract did not effectively control nematode population in Bambara groundnut, but did improve measured plant growth variables.

## CONCLUSIONS

The use of crop varieties exhibiting resistance against pathogenic nematodes is important for limiting nematode populations in soils. Bambara groundnut varieties responded differently to the presence of nematode. All



Bambara groundnut varieties tested were a host to *Meloidogyne incognita* with nematode able to reproduce. Moreover, all tested Bambara groundnut showed a level of tolerance to the nematode. This was observed when the plant growth variables were not affected by the presence of nematode. In this study, the use of these extracts to control nematode population densities in Bambara groundnut was not effective, but NemaCur® effects differed per variety.

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