

Potential use of *Momordica balsamina* ethnomedicinal plant as alternative crop in soil with high population densities of *Meloidogyne incognita* race 2

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Abstract Bitter gourd (*Momordica balsamina*) plant, indigenous to Africa, has various ethnomedicinal practices. A greenhouse study was conducted to determine the plant-nematode relation between *M. balsamina* and *Meloidogyne incognita* race 2. Seven treatments, viz., 0, 250, 650, 1 050, 1 450, 1 850 and 2 250 eggs and second-stage juveniles (J2s) of *M. incognita* race 2, were arranged in a randomised complete block design, with 12 replications. At 56 days after inoculation, the reproductive factor values at all levels of inoculation were less than one, while nematode infection had no effect on yield components of *M. balsamina*. In plant-parasitic nematology, when RF values are less than unity and nematode infection has no effect on growth of the test plant, the plant is resistant to the test nematode. In conclusion, *M. balsamina* was resistant to *M. incognita* race 2 and could therefore be used as an alternative crop for managing population densities of *M. incognita* race 2.

Key words: Indigenous medicinal plants, nematode resistance, relative penetration index

Introduction

Trade in ethnomedicinal plants in South Africa constitutes a multi-billion industry (over R2.9 billion), which represented at least 5.6% of the national health budget (Dold & Cocks, 2002; Anon., 2005; Mander *et al.*, 2005). Consequently, trade in ethnomedicinal plants could be viewed as a potential rural development industry (Moeng & Potgieter, 2011). However, due to the destructive harvesting nature of plants from the wild, certain indigenous ethnomedicinal plants had since become extinct and/or are facing the prospects of extinction (Anon., 2005; Wiersum *et al.*, 2006). Attempts to propagate threatened indigenous ethnomedicinal plants are derailed by the existence of virulent root-knot (*Meloidogyne* species) nematodes (Perry *et al.*, 2009). In addition to the widest host range, the genus has more than 63 species (De Waele & Elsen, 2007), with the highest number of biological races (Robertson & Diez-Rojo, 2008). For instance, the southern root-knot nematode (*Meloidogyne incognita*), has at least six races (Devran & Sogut, 2011; Hartman & Sasser, 1985; Robertson & Diez-Rojo, 2008), which constitute serious challenges in managing the genus using nematode-resistant genotypes. In South Africa, *M. incognita* race 2 is the most widely distributed race in all agro-systems (Kleynhans *et al.*, 1996). Infection by *Meloidogyne* species induces formation of root galls, with various interactive mechanisms resulting in stunted plant growth, decreased water uptake, imbalances of essential nutrient elements, low evapotranspiration and increased root exudation of amino acids (Bird, 1974; Maqbool *et al.*, 1987; Mashela, 2002), all of which may negatively affect the quality of medicines derived from the infected plants. The Cucurbitaceae Family, with 118 genera (Pitrat *et al.*, 1999), contains several genera which are indigenous to

Africa, with potent ethnomedicinal properties. The bitter gourd (*Momordica balsamina* L.) within the family, indigenous to the drier parts of tropical Africa (Gruben & Denton, 2004), has a wide range of potential benefits in ethno- and veterinary-medicines (Hutchings *et al.*, 1996). Leaves, fruits, seeds and bark of *M. balsamina* contain resins, alkaloids, flavonoids, glycosides, steroids, terpenes, cardiac glycoside and saponins (Hutchings *et al.*, 1996). Certain organs of *M. balsamina* were shown to have anti-plasmodial, shigelloidal, anti-diarrheal, anti-septic, anti-bacterial, anti-viral, anti-inflammatory, anti-microbial, hypoglycemic, anti-oxidant, analgesic and hepatoprotective chemical capabilities (Roodt, 1998; Thakur *et al.*, 2009).

In Limpopo Province, South Africa, plants of *M. balsamina* are interplanted with crops for the repellence of the greenhouse whitefly (*Trialeurodes vaporariorum* Westwood) (Mashela, 2012, unpublished data). The greenhouse whitefly is currently an economic pest in tomato, cucumber, lettuce, pepper, potato, strawberry and sweet potato production under both greenhouse and field conditions (Cohen *et al.*, 1992; Winter *et al.*, 1992; Celix *et al.*, 1996; Duffus *et al.*, 1996a,b; Wisler *et al.*, 1998; Tzanetakis, 2004). This whitefly is a viral vector of the genus *Crinivirus*, which contains viruses that belong to the family Closteroviridae, with linear RNA single-strands, renowned for their unusual capacity to multiply rapidly (Tzanetakis, 2004). The listed viruses reduce the longevity and productivity of various crops (Tzanetakis, 2004). During the use of *M. balsamina* as interplants to repel this insect pest, it was observed that although the tomato roots were highly infected by *M. incognita* race 2, roots of *M. balsamina* had no root galls, with subsequent extractions suggesting that there were no nematodes from roots (Mashela, 2012, unpublished data). The nematode-

resistance of *M. balsamina* could be appealing among smallholder farmers for using this plant species as an alternative crop for ethnomedicinal purposes in areas heavily infested with *Meloidogyne* species, more especially because most of the highly effective fumigant nematicides had been withdrawn from the agro-chemical markets due to their environment-unfriendliness (Speth, 2004). Generally, the absence of galls under field conditions does not imply that the plant is resistant to *Meloidogyne* species since it could purely be due to the spatial distribution of nematodes in that particular field. The objective of this study therefore, was to determine the host-status and host-sensitivity of *M. balsamina* to *M. incognita* race 2 under greenhouse conditions.

Materials and methods

The experiments were conducted concurrently in the greenhouse at the Plant Protection Skills Centre, University of Limpopo, South Africa (23°53'10"S, 29°44'15"E) and at the Agricultural Research Council - Industrial Crops Institute, Rustenburg, North West Province (25°43'40"S, 27°17'30"E). Ambient day/night temperatures averaged 29/22°C, with maximum temperatures controlled using thermostatically-activated fans. The trials were conducted in early summer (October – December) 2012 and repeated in late autumn (January–March) 2013.

Ripe fruit were harvested from locally-raised *M. balsamina* plants, cut into pieces, seeds removed and shade-dried for six days. Seeds were sown in seedling trays containing Hygromix growing medium (Hygrotech, Pretoria North, South Africa). Twenty-cm-diameter plastic pots, filled with 2 700 ml steam-pasteurised (300°C for 1 h) river sand and Hygromix at 3:1 (v/v) ratio, were placed on greenhouse benches at 0.3 m inter-row and 0.3 m intra-row spacing, with 5 g 2:3:2 (22) NPK fertiliser mixed into the topsoil of each pot. Uniform four-week-old, nematode-free *M. balsamina* seedlings were transplanted to the pots one day after irrigating the growing medium to field capacity. When required, *M. incognita* race 2 inocula were prepared by extracting eggs and second-stage juveniles (J2s) from roots of greenhouse-grown nematode-susceptible kenaf (*Hibiscus cannabinus* L.) plants in 1% NaOCl solution (Hussey & Barker, 1973).

Seven treatments, viz. 0, 250, 650, 1 050, 1 450, 1 850 and 2 250 eggs and J2s, were arranged in a randomised complete block design, with six replicates. Two days after transplanting, pots were each infested by dispensing approximate numbers of *M. incognita* race 2 eggs and J2s using a 20-ml plastic syringe by placing into 5-cm-deep holes on the cardinal points of the stem of the plants per replication. The zero untreated control plants received filtrate (25- μ m-mesh sieve) of nematode suspension to establish any microbes associated with *Meloidogyne* species in their rhizosphere. Two sets of Hadeco moisture meter were inserted to 20-cm depths in randomly selected pots of each treatment to monitor soil moisture tension. Plants were irrigated with 500 ml tapwater as soon as 50% of the moisture meters have readings just below 2 units.

At 56 days after inoculation, vine length was measured from the terminal end of the short stem to the tips of the vines per plant. Stems were cut at the soil level, shoots were oven-dried for 72 h at 70°C and weighed. Root systems were removed from pots, immersed in water to remove soil particles, blotted dry and weighed to facilitate the calculation of nematode density per total roots per plant. Root galls, when necessary, were assessed using the North Carolina Differential Scale of 0 = no galls, 1 = 1 - 2 galls, 2 = 3 - 11 galls, 3 = 11 - 30 galls, 4 = 31 - 100 galls and 5 = > 100 galls (Taylor & Sasser, 1978). All collected roots were separately weighed. Nematodes were extracted from total roots per plant by maceration and blending for 30 seconds in 1% NaOCl (Hussey & Barker, 1973) and passed through top-down nested 150- μ m and 25- μ m mesh sieves. Contents of the 25- μ m mesh sieve were poured into 100-ml plastic containers for counting under a stereomicroscope. Soil per pot was thoroughly mixed and a 250-ml soil sample was collected. Nematodes were extracted from soil subsamples using the modified sugar-floatation and centrifugation method (Coolen & D'Herde, 1972). Nematode numbers from roots were converted to nematodes per total root system per plant, whereas soil nematode numbers were converted to 2700 ml soil per pot. The reproductive factor (RF = Pf/Pi), described as final population density of nematodes (Pf)/initial population density of nematodes (Pi), were computed.

Data were subjected to analysis of variance (ANOVA) through the SAS software (SAS Institute, Inc., Cary, NC., U.S.A.) to determine the effects of Pi on the RF values and the yield components. Mean separation for significant (P < 0.05) treatments was achieved through the Duncan's multiple-range test. Lines of the best fit were determined for RF values over the $\log_{10}(\text{Pi} + 1)$. Unless otherwise stated, only treatments that were significant at the probability level of 5% were discussed.

Results and discussion

Treatment had significant effect on RF values, contributing 69% to total treatment variation in RF values (data not shown). However, treatment effects were not significant for all plant variables. At all levels of inoculation, RF and RPI values were each less than one (Table 1). Generally, RF values provide an indication of whether a plant is a host or a non-host to the test nematode. The RF values of less than one suggest that the nematode failed to feed and then reproduce in a given plant species (Seinhorst, 1967; Windham & Williams, 1988). Observations in *M. balsamina* confirmed those in other indigenous ethnomedicinal plants, namely, wild watermelon (*Cucumis africanus*) and wild cucumber (*C. myriocarpus*), which were also highly resistant to *M. incognita* race 2 (Pofu *et al.*, 2009; Pofu *et al.*, 2010a,b). Incidentally, the two *Cucumis* species are also in the Cucurbitaceae Family. Suppression of population densities of *M. incognita* race 2 by *M. balsamina* support *in vitro* observations that extracts from the plant were nematocidal to a free-living nematode, *Caenorhabditis elegans* (Beloin *et al.*, 2005)

and that they were also effective in controlling gastrointestinal nematodes in cattle (Amin *et al.*, 2009).

The relationship between the RF values and $\log_{10} (Pi + 1)$ of *M. incognita* race 2 on *M. balsamina* was quadratic – with a downward facing slope (Fig. 1). The observed quadratic relationship suggested that density-dependent growth (DDG) principles were in place (Salisbury & Ross, 1992; Pofu & Mashela, 2013). The DDG principles are depicted through three responses to abiotic and biotic factors, namely, stimulation, neutral and inhibition (Liu *et al.*, 2003). In the current study, all inoculation levels were already beyond the neutral or equilibrium (E) point for *M. incognita* race 2 in *M. Balsamina*, which suggested a low E point relative to the used inoculum levels. This could also be viewed to imply that resistance of *M. balsamina* to *M. incognita* race 2 was quite strong.

In the current study, the aggregated relative penetration index (RPI) was less than one. Pofu & Mashela (2011) developed the RPI to estimate whether resistance was pre- or post-infectious. Generally, in plant-parasitic nematodes forms of resistance are either pre- or post-infectious (Ibrahim *et al.*, 1980; Steele & Savitsky, 1981; Weischer, 1982; Acedo *et al.*, 1984; Huang, 1986; Raja &

Dasgupta, 1986; Kaplan & Davis, 1987). In pre-infectious resistance nematicidal active nematicidal chemicals are released into the rhizosphere, with nematode population densities reduced in the soil (Griffin & Waite, 1971; Richard & DuPree, 1978; Motsinger *et al.*, 2007; Wang *et al.*, 2007). In contrast, in post-infectious resistance, nematodes penetrate the roots, resulting in the activation of passive chemicals – the phytoalexins (Harborne, 1999), which could constitute time-linked resistance (Thurau *et al.*, 2010) or time-unlinked resistance (Wallace, 1973). In our study, the aggregated RPI at all levels of inoculation was less than one, suggesting that the form of resistance was pre-infectious (Pofu & Mashela, 2011). Unlike post-infectious resistance, pre-infectious resistance is not introgressible (Kaplan & Keen, 1988).

Host-sensitivity describes the responses of hosts to nematode infection (Seinhorst, 1967). Generally, when the RF value is less than unity and there is no yield loss, the test plant is said to be resistant (Seinhorst, 1967). Using the Seinhorst model, *M. balsamina* seedlings were therefore, resistant to *M. incognita* race 2. In conclusion, *M. balsamina* is resistant to *M. incognita* race 2, which is widely distributed in various agricultural systems in South

Table 1. Population density (Pf), reproductive factor (RF) and relative penetration index (RPI) of *Meloidogyne incognita* race 2 on *Momordica balsamina* at 56 days after inoculation (n = 72).

Inoculation level (Pi)	Nematode population density (Pf)			Proportion	
	Pf _{soil}	Pf _{root}	Pf _{total}	RF	RPI
250	12	20	32	0.13a	0.67
650	17	23	40	0.06ab	0.35
1 050	33	25	58	0.06ab	-0.24
1 450	53	23	77	0.05ab	-0.57
1 850	12	17	28	0.02b	0.42
2 250	43	20	63	0.03ab	-0.53

$RF = Pf_{total} / Pi$ and $RPI = (Pf_{root} / Pf_{soil}) - 1$.

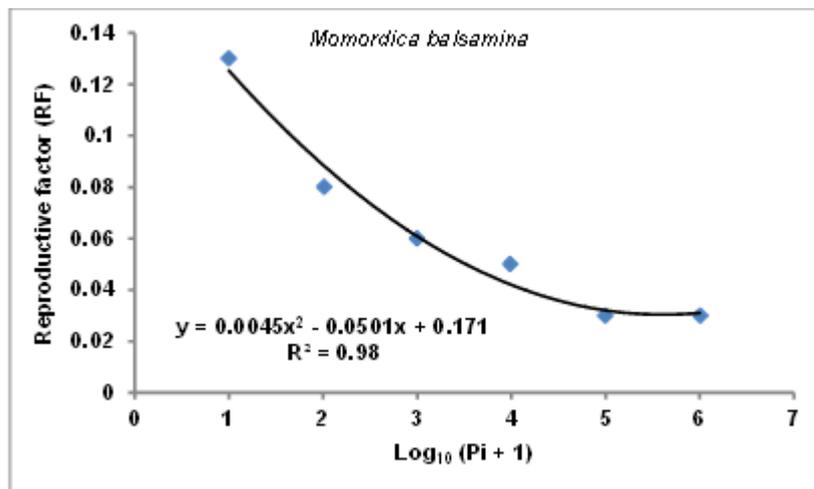


Figure 1. Relationship between the reproductive factor (RF) values and log-transformed initial population density of *Meloidogyne incognita* race 2 on *Momordica balsamina* at 56 days after inoculation (n = 72).

Africa. The observation is important in traditional medicine since it accords *M. balsamina* the status for use as an alternative crop in areas with high population densities of *M. incognita* race 2. Additionally, the observation expands the number of indigenous ethnomedicinal plants in the Cucurbitaceae Family, which could be of use as alternative crops for managing population densities of *Meloidogyne* species for various potential uses.

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