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Potential of cavalcade, *Centrosema pascuorum*, leaves for controlling *Meloidogyne javanica* and quantification of active phytochemicals

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Summary – The legume cavalcade, *Centrosema pascuorum*, is used extensively as a cover crop and as a component of conservation agriculture systems. It is also an attractive rotation or cover crop for the management of root-knot nematodes (RKN; *Meloidogyne* spp.) as it is a non-host. RKN are persistent pests that are well known to be difficult to control. However, the mechanisms governing the non-host status of cavalcade is unknown. The current study established that cavalcade leaves are toxic to RKN as either aqueous extracts or soil amendments. Bioassays conducted using *Meloidogyne javanica* showed that a 90% concentration of aqueous extract derived from 1-month-old cavalcade leaves (89 mg crude extract ml^{-1}) suppressed nematode hatch (82.9%) and killed infective second-stage juveniles of *M. javanica* (85.3%). Soil amendments with 1% (w/w) of 1-month-old cavalcade leaves (0.99 mg crude extract g^{-1} soil) also provided effective control of *M. javanica* in the glasshouse on okra. One-month-old leaves appeared more effective than 2- or 3-month-old leaves. The soil amendments had no adverse phytotoxic effect on okra seed germination. Our study demonstrates the potential for using cavalcade leaves or extracts to manage RKN. This may be due to the nematicidal activity of the various compounds in the leaves, such as flavonoids, phenols and terpenoids, which should be further assessed.

Keywords – cover crop, legume, *Meloidogyne* sp., nematode management, plant extract, root-knot nematode, soil amendment.

Root knot nematodes (RKN), *Meloidogyne* spp., are intractable, soil-borne pests with few management options available to farmers. Among nematode pests, RKN are listed as the most important, while they are also viewed as amongst the most important biotic threats to crop production in the tropics (Jones *et al.*, 2013; Coyne *et al.*, 2018). So far, over 100 known *Meloidogyne* species are associated with damage to horticultural and ornamental crops (Brito *et al.*, 2010; Lopes-Caitar *et al.*, 2019; Rusinque *et al.*, 2022). The most damaging species are *M. arenaria*, *M. incognita*, *M. javanica* and *M. hapla* (Moens *et al.*, 2009) but the list has recently included *M. enterolobii* (Philbrick *et al.*, 2020). A key feature of these species is their polyphagous nature and extensive

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host ranges. *Meloidogyne javanica*, for example, has a worldwide distribution, with over 770 plant species from a wide range of crop types listed as good hosts (Goodey *et al*., 1965; CABI, 2022). In vegetable production, RKN cause 15-85% yield losses, depending on nematode densities and crop (Tuncsoy, 2021). The damage threshold of *M. javanica* on bell pepper is 1.8 eggs and juveniles (g soil)$^{-1}$ at planting (Moosavi *et al*., 2015). In Kenya, *M. javanica* has been detected in several crops, such as bean (Ngundo & Taylor, 1974), cassava (Coyne *et al*., 2006), chickpea (Maina, 2016), famine weed (Chitambo *et al*., 2018), passion fruit (Kanyagia, 1993), pigeon pea (Sharma *et al*., 1993), pineapple (Kiriga *et al*., 2021), potato (Gichure & Ondieki, 1984), pyrethrum (Parlevliet, 1971), sesban (Desaeger & Rao, 2000), sweet potato (Njuguna & Bridge, 1998) and tomato (Birithia *et al*., 2012).

The sustainable management of RKN is difficult to achieve, partly due to their highly polyphagous nature, and partly due to the unspecific above-ground symptoms they are often overlooked or misdiagnosed. Although various management options are available, a combination of methods is undoubtedly necessary for their management, and use of the methods is also dependent on local conditions and circumstances (Viaene *et al*., 2006; Coyne *et al*., 2009; Wendimu, 2021). Whilst there are various management methods available, there is a need to update, adapt and add constantly to the armoury against RKN.

Numerous studies have demonstrated the successful use of plant extracts to control RKN. For instance, aqueous extracts of *Vetiver zizanioides* rendered 70% of *M. incognita* second-stage juveniles (J2) dead (Jindapunnapat *et al*., 2018); aqueous extracts of *Mucuna pruriens*, *Crotalaria spectabilis* and *Dolichos lablab* killed 44, 33 and 41% of J2 of *Meloidogyne* spp., respectively (Osei *et al*., 2010), and aqueous extracts of *Crotalaria* spp. resulted in 41-100% paralysis of *M. incognita*, *M. javanica* and *M. enterolobii* J2 after 48 h exposure (Jourand *et al*., 2004). Besides the use of extracts, leguminous soil amendments or planting as cover crops is being increasingly adopted to suppress RKN. Kankam *et al*. (2015) and Patel & Dhillon (2017) demonstrated that the application of sunn hemp (*C. juncea*) as a soil amendment decreased *M. incognita* densities by up to 94%. Jindapunnapat *et al*. (2019) found that incorporating 10% fresh *V. zizanioides* into the soil reduced *M. incognita* infection of cucumber by 46-67%. In Cambodia, *C. pascuorum* and *Stylosanthes guianensis* 'Nina' have been used as cover crops under conservation agriculture in rice fields, resulting in a decrease of *Hirschmanneilla* spp. and *M. graminicola* root densities (Beesa *et al*., 2021; Masson *et al*., 2022).

The nematicidal compounds from numerous plant species have received extensive attention to date (*e.g.*, Chitwood, 1992; Chen & Song, 2021) with numerous identified, *viz*. polythienyls, alkaloids, acetylenes, quassinoids, fatty acids and derivatives, miscellaneous phenolics, flavonoids, terpenoids, post-infectional compounds, and miscellaneous ketones and aldehydes. Wang *et al*. (2002) reported that *C. juncea* generated allelochemicals such as pyrrolizidine, alkaloids and monocrotaline that are toxic to *Meloidogyne* spp. Yang *et al*. (2015) also analysed compounds from *Camellia* spp. seed cake extracts by GC-MS and found that 4-methylphenol exhibited high nematicidal activity. In addition, Rocha *et al*. (2017) determined the nematicidal compounds from aqueous crude extracts of *Canavalia ensiformis* as D-glucose, L-canavanine, xanthotoxin, cis-aconitic acid, trans-aconitic acid, malic acid, citric acid, palmitic acid and S-carboxymethylcysteine.

Cavalcade (*Centrosema pascuorum*) is a leguminous crop belonging to the Family Fabaceae (Clements, 1984) originating from tropical South and Central America (Clements, 1984). Cavalcades are mainly used as a hay crop to feed cattle (Cameron, 2005). Recently, Suong *et al*. (2019) and Beesa *et al*. (2021) demonstrated the yield benefits to rice when simultaneously planted with cavalcade and *S. guianensis* in Cambodia. The improved rice yields likely resulted from the higher nutrient and organic carbon availability, but also possibly from the nematode management, as cavalcade has been shown to act as a non-host to *M. graminicola* (Suong *et al*., 2019). However, no further information appears to exist on the nematode management potential of cavalcade. Therefore, the current study was conducted to evaluate using cavalcade leaves and aqueous extracts as soil amendments against *M. javanica*. Some of the phytochemicals contained in cavalcade leaves, including flavonoids, phenols and terpenoids, were also quantified.

Materials and methods

Nematode preparation

The RKN population used in the study was collected from infected tomato plants at a Juja farm in Kiambu country, Kenya (1°09′37.1″S, 37°04′13.5″E). Egg masses on the tomato plants were removed manually and inoculated onto 10-day-old okra seedlings grown in pots filled with 1:1 (w/w) sterilised soil and sand to multiply and

maintain the nematode inoculum. To extract eggs and J2, 2-month-old infected okra roots were cut into small pieces (0.5-1.0 cm long), placed into 50 ml Falcon tubes filled with 30 ml of 0.6% NaOCl and shaken by hand for 2 min 30 s (Meyer *et al.*, 2020; Beesa *et al.*, 2022). The nematode suspension was poured through nested 90 and 25 μm aperture sieves and rinsed under running tap water until clean. The J2 and eggs, collected on a 25 μm aperture sieve, were incubated at 27°C for 7 days for use as inoculum.

Nematode identification

Morphological identification by perineal pattern

Thirty adult RKN females were removed from the cultured okra roots for use to identify to species by perineal pattern analysis. Briefly, one adult female was transferred to a drop of water and cut using a razor blade to separate the anterior and posterior parts on a glass slide under a stereomicroscope (HSZ-ZB700, Huvitz). The perineal areas were transferred to a drop of 45% lactic acid for trimming and cleaning and then to a drop of distilled water mounted on a glass slide, covered with a coverslip, and sealed with nail polish. The slide was labelled and placed under a compound microscope (Leica DM750) for morphological observation according to Hartman & Sasser (1985).

Molecular identification

For molecular identification, seven adult RKN females were extracted from infected okra plants. The DNA was extracted using the method of Holterman *et al.* (2006) with slight modifications. In brief, one adult female was placed in a 0.5 ml PCR tube filled with 20 μl distilled water. Then, 20 μl lysis buffer (200 mM NaCl, 200 mM Tris-HCl pH 8.0, 1% (v/v) β-mercaptoethanol (all Sigma-Aldrich), and 800 μg ml^{-1} proteinase K (Meridian Bioscience)) was added and the mixture incubated at 65°C for 90 min, followed by 7 min at 99°C in a PCR Thermocycler (ProFlex, PCR System). The extracted DNA from each sample was stored at –20°C until use.

The PCR was performed using three primer sets: JB3/JB4.5 (5′-TTTTTTGGGCATCCTGAGGTTTAT-3′/5′-TAAAGAAAGAACATAATGAAAATG-3′) for cytochrome *c* oxidase subunit 1 (*COI*) mitochondrial gene (Bowles *et al.*, 1992; Derycke *et al.*, 2010), D2A/D3B (5′-ACAAGTACCGTGAGGGAAAGTTG-3′/5′-TCGGAAGGAACCAGCTACTA-3′) for partial 28S rRNA gene (Nunn, 1992) and Fjav/Rjav (5′-GGTGCGCGATTGAACTGAGC-3′/5′-CAGGCCCTTCAGTGGAACTATAC-3′) for species-specific primer (Zijlstra *et al.*, 2000). A 20 μl PCR reaction included 3 μl of DNA template, 12 μl of sterilised distilled water, 0.5 μl each of 10 μM forward and reverse primers and 4 μl of 5×HOT FIREPol Blend Master Mix (Solis BioDyne). The PCR condition was performed as follows: D2A/D3B; denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 1 min, and final extension at 72°C for 5 min, JB3/JB4.5; 94°C for 7 min, 5 cycles of denaturation at 94°C for 30 s, annealing at 54°C for 30 s and temperature decreasing with 1°C for each cycle, and extension at 72°C for 30 s, followed by 35 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 30 s, and a final extension of 10 min at 72°C and Fjav/Rjav; denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 64°C for 30 s and 72°C for 1 min, and final extension at 72°C for 7 min. Four representative samples of PCR products for each primer set were purified by rSAP and Exo I (BioLabs) and then sent to Macrogen Inc, Korea, for DNA sequencing. The obtained nucleotide sequences were aligned using ClustalW. Then, the aligned sequences were compared and deposited in the NCBI GenBank database.

Plant material and aqueous extract preparation

Cavalcade plants were grown in 25-cm-diam. pots filled with 1 kg sterilised paddy soil and maintained in a glasshouse, Faculty of Agriculture, Kasetsart University, at 25-33°C. Fertiliser (N-P-K, 16-16-16) was applied twice at 3 and 6 weeks after transplanting (3 g pot^{-1}). Subsequently, 1-, 2- and 3-month-old cavalcade leaves were harvested, rinsed under running tap water and air-dried in the shade for 14 days. The dried plant materials were ground to fine particles in a blender (DXM-1000, DXFILL machine) and kept at room temperature until use.

To prepare aqueous extracts, 10 g of each ground material was added to 100 ml of distilled water in a 250 ml conical flask and shaken on an incubator shaker series (Innova44; New Brunswick Scientific) at 100 rpm for 24 h at 25°C. The mixtures were filtered through muslin cloths, centrifuged at 3000 *g* for 10 min, and the supernatants filtered through 0.45 μm and 0.22 μm syringe filters (Cole-Parmer) (Meyer *et al.*, 2006). The obtained extracts were held at 4°C until use as stock solution (100% concentration) in microwell assays.

Second-stage juveniles hatching and mortality tests

Second-stage juvenile hatching and mortality assays with cavalcade leaf aqueous extracts were conducted in 96-well polystyrene plates, according to the procedures of Jindapunnapat *et al*. (2018). A 15 μl nematode suspension containing approximately 30 $\pm$ 5 eggs or J2 was added to each well in 185 μl of each diluted aqueous extract (25, 50 and 90% at final concentration). Streptomycin sulphate (Sigma-Aldrich) was used to prevent bacterial contamination. To prepare diluted aqueous extracts (185 μl), a stock solution (100%) was diluted by distilled water to give three concentrations: *i*) 25% = 50 μl of stock solution + 133 μl of distilled water + 2 μl of 50 mg ml^{-1} streptomycin sulphate; *ii*) 50% = 100 μl of stock solution + 83 μl of distilled water + 2 μl of 50 mg ml^{-1} streptomycin sulphate; and *iii*) 90% = 180 μl of stock solution + 3 μl of distilled water + 2 μl of 50 mg ml^{-1} streptomycin sulphate. Distilled water (0%) with 0.5 mg ml^{-1} streptomycin sulphate served as controls. The plates were covered with cling film and incubated at 27°C. The experiment included two factors and was arranged in a randomised completely block design (RCBD) with five replications and repeated once. The two factors were: *i*) age of cavalcade leaves; and *ii*) rate of application. The study included eleven treatments: 1) distilled water; 2) 0.5 mg ml^{-1} streptomycin sulphate; 3) 25% of 1-month-old cavalcade leaves (1MAE); 4) 50% of 1MAE; 5) 90% of 1MAE; 6) 25% of 2-month-old cavalcade leaves (2MAE); 7) 50% of 2MAE; 8) 90% of 2MAE; 9) 25% of 3-month-old cavalcade leaves (3MAE); 10) 50% of 3MAE; and 11) 90% of 3MAE. For hatching tests, the number of J2 that hatched was recorded at 7 days after exposure. For J2 mortality, inactive J2 were counted, after being rinsed three times with distilled water, at 2 days after immersion. At 72 h after incubation (3 days), dead J2 were re-checked. If the nematodes were not active they were considered dead. The percentage inhibition of hatching (Talavera-Rubia *et al*., 2020) and the percentage J2 mortality was calculated.

Glasshouse experiments

Preventive treatments

This study was conducted in the glasshouse at *icipe* campus located in Nairobi, Kenya, between July and September 2022. The daily temperature ranged between 11 and 28°C (AccuWeather, 2022). The powder of 1-, 2- and 3-month-old cavalcade leaves was mixed with 200 g of 1:1 (w/w) sterilised sand and soil (air-dried soil) with 0, 0.2, 0.5 and 1% (w/w) and filled in 7 cm diam. plastic pots. The mixtures were watered daily with 20 ml of tap water and incubated in the glasshouse for 6 days. Ten-day-old okra seedlings were rinsed free of soil under the tap and transplanted singly into each pot. Five days later, 500 eggs + J2 were inoculated in 1.5 ml water suspension into three 1.5 cm deep holes made around each plant using a pencil. Fertiliser (N-P-K, 16-16-16) was applied once at 7 days after nematode inoculation (0.1 g pot^{-1}). The experiment included two factors and was arranged in a RCBD with four replications and repeated once. The two factors were: *i*) age of cavalcade leaves; and *ii*) rate of application. Data for fresh root weights, number of galls and number of egg masses (staining with phloxine B) were recorded at 5 weeks and 6 weeks after nematode inoculation for the two trials, respectively.

Eradicative treatments

This experiment was conducted using the same treatments and conditions as above, but the nematodes were inoculated into amended soil 5 days before transplanting okra seedlings. The fertiliser (N-P-K, 16-16-16) was applied once at 7 days after okra transplantation (0.1 g pot^{-1}). Plants were harvested and data on root fresh weights, number of galls and egg masses were determined as for the preventive treatment experiment.

Quantification of phytochemicals

Aqueous crude extracts of 1-, 2- and 3-month-old cavalcade leaves were prepared as above. The crude extracts were freeze-dried for 5 days using a Wizard 2.0 freeze-dryer (SP Scientific). Each cavalcade leaf age was replicated six times. The obtained crude extracts were weighed and kept at 4°C until use.

Total flavonoid (TFC), phenol (TPC) and terpenoid (TTC) contents were quantified according to Kegode *et al*. (2022). A 0.25 g aqueous crude extract were each dissolved in 2.5 ml of 50% methanol. The obtained crude extracts were used to determine the following phytochemical contents. Each analysis of phytochemicals was carried out in triplicate.

Flavonoid content was determined using the aluminium chloride ($AlCl_3$) colorimetric assay described by Popova *et al*. (2005). Briefly, 1 ml of obtained extract was mixed with 4 ml of distilled water and 300 μl of 5% (w/v) $NaNO_2$ and incubated for 5 min. Then 300 μl of 10% $AlCl_3$ was added and left for 1 min prior to adding 2 ml of 1 M NaOH and 2.4 ml of distilled water at the final step. The mixtures were centrifuged at 1300 *g* for 2 min. The total flavonoid content was determined

by measuring the absorbance at 510 nm using a UV spectrophotometer (BioSpec). Quercetin (QE) at different concentrations (20-200 μg ml^{-1}) served as a standard ($y = 0.0006x + 0.0028$, $R^2 = 0.9981$) and calculated and expressed as mg quercetin equivalent per g crude extract (mg QE g^{-1}).

Phenol content was established using the Folin–Ciocalteu method (Popova *et al.*, 2005). In brief, 5 ml of 0.2 M Folin-Ciocalteu reagent was added to 1 ml of extract and left at room temperature for 5 min before adding 4 ml of 75 g l^{-1} Na_2CO_3 and leaving for a further 1 h. The mixtures were then centrifuged at 1300 *g* for 2 min. The absorbance of the mixture was measured at 760 nm in a UV spectrophotometer. Gallic acid (GAE) at different concentrations (0-250 μg ml^{-1}) served as a standard ($y = 0.0073x + 0.0233$, $R^2 = 0.999$). The total phenol content was calculated and expressed as mg gallic acid equivalent per g crude extract (mg GAE g^{-1}).

Terpenoid content was determined using the colorimetric method (Malik *et al.*, 2017) by adding 1.5 ml chloroform to 200 μl crude extract, and thoroughly mixing using vortex for 1 min. Then 100 μl of concentrated H_2SO_4 was added and incubated in the dark for 1 h at room temperature. The supernatant was gently removed, leaving the reddish-brown precipitate at the bottom and 1.5 ml of absolute methanol added and mixed using vortex to dissolve the precipitate. The mixtures were centrifuged at 1300 *g* for 2 min and the terpenoid content estimated from the standard curve plotted using 0-500 mg ml^{-1} linalool (LE) ($y = 0.0009x - 0.0158$, $R^2 = 0.9914$). The absorbance was measured at 538 nm in a UV spectrophotometer with methanol as the blank. The total terpenoid content was calculated and expressed as mg LE acid equivalent per g crude extract (mg LE g^{-1}).

Phytotoxicity assessment on okra

Soil amendment with cavalcade leaves was investigated for phytotoxicity on okra seed germination in the glasshouse at the *icipe* campus in Nairobi, Kenya. The pounded dry cavalcade leaves preparation (1-, 2- and 3-months-old) were each assessed at four rates: 0, 0.2, 0.5 and 1% (w/w), by thoroughly mixing with 60 g sterilised soil (1:1 (w/w) soil:sand) in 9 cm diam. Petri dishes with ten okra seeds placed at approximately 0.5 cm depth and incubated for 6 days. The experiment included two factors and was arranged in a RCBD with five replications and repeated once. The two factors were: *i*) age of cavalcade leaves; *ii*) rate of application. Each Petri dish was irrigated with 10 ml tap water daily until the termination of the experiment at 5 days after planting. Seed germination (G) and radical length (RL) of okra sprouts were recorded and the G and RL values used to calculate seed germination index (GI):

$$\mathrm{GI} = \left[\frac{\text{RL of treatment} \times \text{G of treatment}}{\text{RL of control} \times \text{G of control}}\right] \times 100$$

The mean GI values were compared to phototoxicity scores (Barral & Paradelo, 2011) as follows: GI $< 50\%$ represents high phytotoxicity; GI 50-80% represents moderate phytotoxicity; GI $> 80\%$ represents no phytotoxicity; and GI $> 100\%$ represents capability to be a phytonutrient.

Statistical treatment of data

All data were presented as means $\pm$ standard error. The data were statistically analysed using the SPSS software (version 16.0; SPSS). Differences among means were determined by Tukey's multiple comparison ($P \leqslant 0.05$). In this study, a two-way ANOVA test was carried out to compare the main factor and interaction effects of leaf age (Age) and concentration (Dose).

Results

Nematode identification

The perineal patterns of all RKN females assessed were round or oval-shaped, with moderately high dorsal arch and obvious double lateral lines, which matched with the descriptions of *M. javanica* (Hunt & Handoo, 2009). The DNA amplification resulted in a specific amplicon size of 400, 750 and 670 bp for the three primer sets, which gave a 98-99% similarity to *M. javanica* sequences available in GenBank, confirming the identity as *M. javanica*. All sequences were deposited in GenBank under the accession numbers OP646643-OP646646 for *COI*, OP802558- OP802561 for D2-D3 and OP972598-OP972601 for the species-specific primers.

Hatching test

The effect of leaf age and dosage on hatching of *M. javanica* was analysed using a two-way ANOVA. The 50% and 90% concentrations of all cavalcade leaf ages were effective at reducing the hatch of *M. javanica* after immersion for 7 days (Fig. 1). By contrast, the 25% concentration of all leaf ages did not inhibit hatch when compared with the water and 0.5 mg ml^{-1} streptomycin sulphate controls. Maximum hatch inhibition (82.9%)

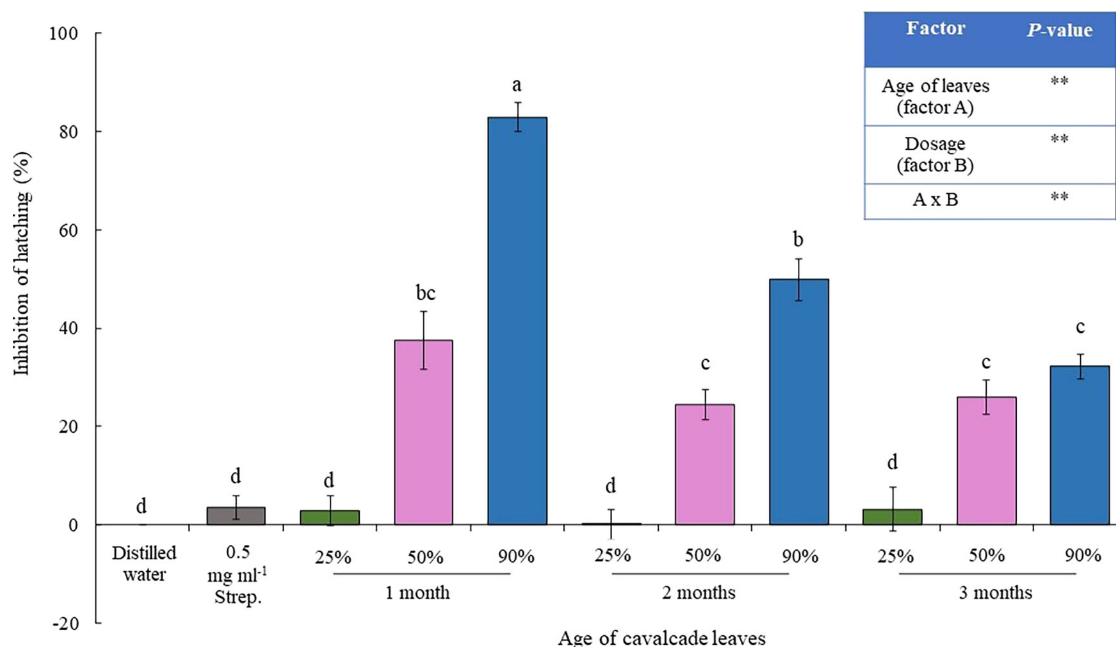

Fig. 1. Effect of aqueous extracts of cavalcade leaves at different ages (factor A) and dosages (factor B) on percentage inhibition of *Meloidogyne javanica* hatch. Bars refer to standard error of the mean from two trials (n = 10). Similar lower-case letters in each value indicated that means are not significantly different according to Tukey's multiple comparison at 0.05 level. **Significant at the 0.01 level.

was observed with a 90% concentration derived from 1MAE, followed by 90% 2MAE (49.9%) and 50% 1MAE (37.5%). At the 50% concentration, hatch was similar among different leaf ages. However, at the 90% concentration, younger leaf age (1MAE) showed higher potency in inhibiting hatch, as compared to the older leaf ages (2MAE and 3MAE).

Juvenile mortality test

The two-way ANOVA indicated that there was an interaction between the age of leaves and the dosage on percentage J2 mortality at 72 h after immersion. The 25% concentration for any leaf age had no adverse effects on J2. However, the 50% and 90% concentrations derived from 1-month-old leaves (1MAE) caused markedly greater J2 mortality than the same concentrations from 2- and 3-month-old leaves (2MAE and 3MAE). At 48 h after immersion, inactivity of J2 reached 88.5-100% for all concentrations across all leaf ages, compared with the water (1.2%) and streptomycin sulphate (3.4%) controls (Table 1). However, at 72 h, over half of the J2 resuscitated from their inactive state, across treatments. The greatest inhibitory effects on J2 (85% mortality) were recorded for 90% 1MAE followed by 90% 2MAE (55.8%), 90% 3MAE (52.7%) and 50% 1MAE (47.4%).

Glasshouse experiments

No phytotoxic effects were observed in either of the two experiments (preventive or eradicative) (Table 2; Fig. 2). In addition, interactions were observed in amended rates (factor B) on suppressing *M. javanica* in both preventive and eradicative treatments. The 0.5% and 1% amended rates of cavalcade leaves considerably reduced the number of galls (g root)$^{-1}$ and egg masses (g root)$^{-1}$. RKN suppression increased with increasing rate of cavalcade leaf concentration. The 1% 1-month-old (both preventive and eradicative treatments) leaves was the most effective at reducing *M. javanica* damage.

Quantification of phytochemicals

The weight of aqueous crude extracts derived from 1 g dried cavalcade leaves revealed that crude extracts were similar between the three different leaf ages, although there was a relatively higher quantity in 1-month-old

Table 1. Percentage inactivity and mortality of *Meloidogyne javanica* infective second-stage juveniles (J2) after exposure to different concentrations and ages of cavalcade leaves.

Age of leaves	Dosage	Hours after exposure	
		48 h (% inactive J2)	72 h (% dead J2)
Control	Distilled water	1.2 ± 0.6d[1]	1.5 ± 0.8e
	0.5 mg ml^{-1} Strep. sulphate	3.4 ± 0.9d	2.9 ± 1.1e
1 month	25%	89.9 ± 3.7c	10.9 ± 2.3de
	50%	98.4 ± 1.6ab	47.4 ± 2.7b
	90%	100a	85.3 ± 2.6a
2 months	25%	88.5 ± 4.1c	4.2 ± 1.1e
	50%	99.6 ± 0.4a	24.4 ± 3.6c
	90%	100a	55.8 ± 3.4b
3 months	25%	91.8 ± 1.6bc	7.6 ± 1.9e
	50%	99.6 ± 0.4a	19.5 ± 2.8cd
	90%	100a	52.7 ± 3.7b
P value			
Leaf age		ns	**
Dosage		**	**
Leaf age × Dosage		ns	**

[1]Values are means ± SE of five replications from two trials (n = 10). Means were compared by Tukey's multiple comparison at 0.05 level. Similar lower-case letters in each column indicated that means are not significantly different.
**Significant at the 0.01 level.

(98.89 mg) leaves. The information obtained reflects the tested concentrations of the above experiments as follows: bioassays with 90% of 1MAE, 2MAE and 3MAE were 89, 86 and 80 mg ml^{-1}, respectively, and soil amendments with 1% of 1-, 2- and 3-month-old leaves were 0.99, 0.96 and 0.89 mg (g soil)$^{-1}$, respectively.

Of the three phytochemical groups, higher concentrations of flavonoids (26.8 mg QE g^{-1}) and phenols (2.35 mg GAE g^{-1}) were detected in aqueous cavalcade leaf crude extracts from 1-month-old leaves (Fig. 3). However, the phenol content was not significantly different from 3-month-old leaves (2.25 GAE g^{-1}), while terpenoid contents were lower in 1-month-old (90.4 mg LE g^{-1}) than 2- (133 mg LE g^{-1}) and 3-month-old (131.4 mg LE g^{-1}) leaves.

Phytotoxicity assessment on okra

The radicle length of okra seedlings and the percentage seed germination were unaffected following soil amendment with dried cavalcade leaves (Table 3). No adverse effects on okra seedlings were observed with GI values ranging from 81.2 to 104.6%, portraying no phytotoxicity. Moreover, soil amendment with 3-month-old cavalcade leaves appeared to act as a phytonutrient. No interaction between the age of cavalcade leaves and rate of application was observed to influence the percentage seed germination. Therefore, any leaf ages or amended rate uses as soil amendments produced the same results, which did not pose negative effects to seed germination.

Discussion

This current study demonstrates the potential for the leguminous crop *C. pascuorum* to contribute to the management of RKN. Although experiments were mostly *in vitro*, results clearly showed effective inhibition of hatch and J2 mortality of *M. javanica*, leading to reduced root galling damage and egg mass production under glasshouse conditions. Our study further revealed that fresher or younger leaf age appeared to be more potent with higher inhibitory effects on the nematode. This may be due to the loss of nematicidal compounds, such as flavonoids, phenols and terpenoids, with age of the *C. pascuorum* leaf extracts. Such volatile compounds are known to play an important role against *M. javanica* (Chitwood, 1992). The higher concentrations of the extracts also corresponded with higher nematode suppression, while no negative phytotoxicity effects were observed at the

Table 2. Effect of dried cavalcade leaf tissue of three different ages (1, 2 and 3 months) incorporated into soil at three rates on okra fresh root weights, number of galls and egg masses of the root-knot nematode (RKN) *Meloidogyne javanica* in a glasshouse in Kenya.

Age of leaves	Amended rates	Fresh root weight (g)		No. of galls (g root)$^{-1}$		No. egg masses (g root)$^{-1}$	
		Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Preventive treatments							
Control	0%, −RKN	0.4 ± 0.1b[1]	0.4 ± 0.1a	0d	0c	0d	0d
	0%, +RKN	0.7 ± 0.1ab	0.5 ± 0.1a	102.6 ± 7.3a	122.6 ± 10.0a	11.2 ± 1.1a	16.0 ± 1.5a
1 month	0.2%	0.9 ± 0.1ab	0.4 ± 0.1a	82.1 ± 9.3abc	106.1 ± 15.7ab	7.1 ± 1.2abc	9.3 ± 0.7b
	0.5%	0.7 ± 0.1ab	0.5 ± 0.0a	84.2 ± 3.1ab	84.0 ± 9.5ab	5.6 ± 0.3bc	7.4 ± 2.2bc
	1%	0.7 ± 0.1ab	0.4 ± 0.0a	70.2 ± 9.5bc	63.8 ± 11.5b	4.1 ± 1.1cd	2.7 ± 1.0cd
2 months	0.2%	0.9 ± 0.0a	0.5 ± 0.1a	67.8 ± 4.5bc	110.4 ± 12.1ab	8.9 ± 0.9ab	10.4 ± 1.0b
	0.5%	0.7 ± 0.1ab	0.5 ± 0.1a	64.6 ± 8.0bc	75.6 ± 7.7ab	5.6 ± 1.6bc	2.8 ± 1.0cd
	1%	0.6 ± 0.1ab	0.5 ± 0.0a	65.6 ± 4.9bc	85.8 ± 9.0ab	6.5 ± 0.6bc	5.9 ± 0.6bc
3 months	0.2%	0.8 ± 0.1ab	0.7 ± 0.0a	81.1 ± 6.6abc	105.8 ± 6.6ab	8.6 ± 0.8abc	10.8 ± 0.8b
	0.5%	0.6 ± 0.1ab	0.6 ± 0.1a	52.1 ± 5.6c	98.2 ± 13.9ab	7.5 ± 1.2abc	5.6 ± 0.9bc
	1%	0.7 ± 0.1ab	0.6 ± 0.1a	64.4 ± 5.3bc	82.4 ± 10.3ab	4.0 ± 1.3cd	6.8 ± 1.0bc
Eradicative treatments							
Control	0%, −RKN	1.0 ± 0.1a	1.1 ± 0.1a	0c	0c	0c	0c
	0%, +RKN	0.9 ± 0.0a	0.9 ± 0.0a	56.9 ± 7.7a	34.1 ± 3.7ab	7.0 ± 1.1a	6.4 ± 0.8a
1 month	0.2%	0.9 ± 0.1a	1.0 ± 0.2a	34.3 ± 10.1ab	29.2 ± 6.4ab	5.7 ± 1.0ab	5.1 ± 1.1abc
	0.5%	0.8 ± 0.1a	1.0 ± 0.1a	39.4 ± 9.2ab	16.2 ± 4.4bc	2.5 ± 1.1bc	2.6 ± 1.0abc
	1%	0.8 ± 0.1a	0.9 ± 0.0a	9.3 ± 3.6bc	3.4 ± 0.7c	0.9 ± 0.6c	0.3 ± 0.3bc
2 months	0.2%	1.0 ± 0.0a	1.1 ± 0.1a	34.0 ± 4.1ab	40.5 ± 4.4a	2.9 ± 0.6bc	8.5 ± 1.8a
	0.5%	0.9 ± 0.1a	1.0 ± 0.1a	33.9 ± 6.4ab	33.4 ± 6.5ab	3.1 ± 0.7bc	3.7 ± 1.2abc
	1%	1.0 ± 0.1a	1.2 ± 0.1a	22.5 ± 2.3bc	31.6 ± 9.5ab	0.8 ± 0.5c	4.7 ± 1.9abc
3 months	0.2%	0.8 ± 0.0a	1.0 ± 0.0a	32.9 ± 6.4ab	29.8 ± 3.6ab	3.8 ± 0.9abc	6.0 ± 1.0ab
	0.5%	0.9 ± 0.0a	1.2 ± 0.1a	30.8 ± 7.3ab	27.9 ± 1.9ab	2.0 ± 0.6bc	4.4 ± 0.5abc
	1%	0.8 ± 0.1a	1.1 ± 0.0a	38.1 ± 6.6ab	38.5 ± 2.7ab	3.9 ± 1.2abc	5.5 ± 2.0abc
P values							
Preventive treatments							
Leaf ages		ns	ns	ns	ns	ns	ns
Amended rates		**	ns	**	**	**	**
Leaf ages × Amended rates		ns	ns	ns	ns	ns	ns
Eradicative treatments							
Leaf ages		ns	ns	ns	**	ns	*
Amended rates		ns	ns	**	**	**	**
Leaf ages × Amended rates		ns	ns	ns	**	ns	ns

[1] Values are means ± SE of four replications (n = 4). Means were compared by Tukey's multiple comparison at 0.05 level. Similar lower-case letters in each column indicated that means are not significantly different.

*, ** Significant at the 0.05 and 0.01 levels, respectively.

application rates used in this study. Our study reflects results from a separate study, using the leguminous cover crops *C. pascuorum* and *S. guianensis* that suppressed *Hirschmanniella* spp. population densities in rice fields (Suong *et al.*, 2019; Beesa *et al.*, 2021). However, to date no reports have described the governing mechanism of *C. pascuorum* for controlling the nematodes.

The highest concentration (90%) 1 MAE of the aqueous extracts of cavalcade leaves showed the greatest inhibitory effects on *M. javanica* and the aqueous extracts derived

Fig. 2. Okra roots grown in soil amended with 1% cavalcade leaves and inoculated with the root-knot nematode (RKN) *Meloidogyne javanica*. A: Preventive treatments: seedlings inoculated 5 days after transplanting; B: Eradicative treatments: seedlings inoculated 5 days before transplanting.

from 1-month-old cavalcade leaves displayed more effective reduction of *M. javanica* than those obtained from 2- and 3-month-old leaves, indicating that there is a loss of potency with age. Similar results were reported by Jindapunnapat *et al*. (2018), who noted that aqueous root and shoot extracts derived from younger plants (2 months old) of *V. zizanioides* showed greater activity against *M. incognita* J2 than older plants (4 years old). Other studies have also demonstrated that aqueous extracts of leguminous leaf material control RKN, such as *Mucuna pruriens*, *Crotalaria spectabilis* and *Dolichos lablab* (Jourand *et al*., 2004; Osei *et al*., 2010). Aqueous extracts from *Cajanus cajan*, *Crotalaria* and *C. cajan* + *Crotalaria* also led to 100% nematicidal effect on *Scutellonema bradys* (Garrido *et al*., 2008), while extracts from a range of legume crops have conversely been shown to possess no nematicidal activity, including water extract of *Crotalaria* seeds (Harender *et al*., 2012). With regard to the use of cavalcade aqueous leaf extracts, the current study is the first such study to assess and document its potential for nematicidal activity against RKN, specifically *M. javanica*.

The use of cavalcade leaves as a soil amendment to protect plants against RKN was also demonstrated in the current study. The use of the youngest (1-month-old) leaves showed greatest impact on *M. javanica* infection of okra in the glasshouse. Although all treatments reduced the number of galls and egg masses on roots, the 1% amended rate of 1-month-old leaves were most effective. This result reflects studies by Kankam *et al*. (2015) and Patel & Dhillon (2017) using sunn hemp (*C. juncea*) as a soil amendment, which significantly decreased *M. incognita* densities by 51-94%. Another study found that incorporation of 10% fresh vetiver into the soil significantly reduced *M. incognita* by 46-67% (Jindapunnapat *et al*., 2019). The use of cavalcade and *S. guianensis* ('Nina') as cover crops in rice fields in Cambodia has been associated with reduced densities of *M. graminicola* and *Hirschmaniella* sp. (Beesa *et al*., 2021; Masson *et al*., 2022). Our current study confirms the suppressive effect of cavalcade and indicates that it is likely due to nematotoxic compounds. In terms of the effect of cavalcade leaf amendment on seed germination, no negative impact was observed on okra seed germination. Moreover, soil amended with 3-month-old cavalcade leaves appears to act as a phytonutrient. Similar results have been reported by Moldes *et al*. (2007), who recorded GI value > 125 (act as phytonutrient) of rye grass in composted grape pomace. Islam & Faruq (2008) also found that soil amended with neem compost significantly increased tomato seed germination (29.85%), root length (48.22%) and shoot length

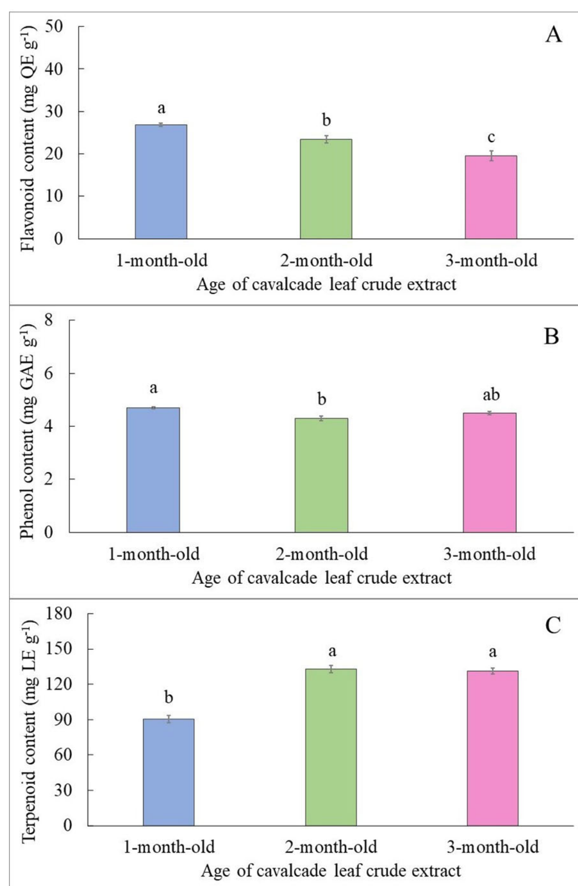

Fig. 3. Quantification of flavonoid (A), phenol (B) and terpenoid (C) contents derived from aqueous crude extracts of 1-, 2- and 3-month-old cavalcade leaves. Bars refer to standard error of the mean (n = 3). Similar lower-case letters in each figure indicated that means are not significantly different according to Tukey's multiple comparison at 0.05 level. QE = Quercetin Equivalent, GAE = Gallic Acid Equivalent, LE = Linalool Equivalent.

(72.25%). When using *S. guianensis* and *C. pascuorum* as cover crops, soil fertility (soil organic matter) and rice yields in Cambodian were shown to increase (Suong *et al*., 2019; Beesa *et al*., 2021).

In our study, the greatest nematicidal activity was observed with the youngest leaf material, which may be associated with higher bioactive compounds in crude extracts of flavonoids and phenols. The presence of flavonoid, phenol and terpenoid phytochemicals are likely associated with nematicidal activity, as indicated in other studies (Chitwood, 1992; Chen & Song, 2021). Several flavonoid compounds, *e.g.*, flavonols, kaempferol, quercetin, and myricetin, are also known to repel RKN J2 and to inhibit hatch (Wuyts *et al*., 2006), due to slowing the movement of J2, modifying nematode orientation to the plant roots and killing nematodes (Chin *et al*., 2018). From 49 phenolic compounds screened against *M. incognita*, D-(−)-4-hydroxyphenylglycine, *t*-butylhydroquinone, L-3-(3,4-dihydroxyphenyl) alanine, sesamol, 2,4-dihydroxyacetophenone, and *p*-anisaldehyde were found to be lethal to RKN J2 (Oliveira *et al*., 2019), whilst various studies have shown the nematicidal effect of a range of phenolic compounds (Ohri & Pannu, 2010) and other volatiles (Abdel-Rahman *et al*., 2013). Echeverrigaray *et al*. (2010) similarly showed significant *M. incognita* hatch reduction by monoterpenoids and reduced J2 mobility at a concentration of 0.25 mg ml^{-1}. Therefore, this study reveals that the nematicidal activity of cavalcade leaves on *M. javanica* could be associated with the presence of flavonoid, phenolic and terpenoid compounds. However, additional studies are needed to clarify the nematicidal compounds present in cavalcade leaves that are responsible for controlling *M. javanica*.

In conclusion, our study provides strong evidence on the nematicidal effects of cavalcade and its potential as a bio-based option for nematode management. In the current study, it was demonstrated that aqueous extracts of dried leaves achieved high efficiency in controlling *M. javanica* in both *in vitro* and glasshouse settings. The controlling mechanism may be associated with the existence of nematicidal flavonoid, phenol and terpenoid volatile compounds that appear to reduce in potency with age of the leaves. More extensive research is naturally required to determine further the real potential of cavalcade for nematode management on a field basis. However, this study provides a sound and promising basis upon which to build.

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Table 3. Influence of cavalcade leaf age and concentration on okra seed germination under glasshouse conditions in Kenya.

Age of leaves (Factor A)	Amended rates (Factor B)	Radical length (cm)	Germination (%)	Germination index (GI)	Phytotoxicity reaction
Control	0%	1.9 ± 0.1ab[1]	94.0 ± 2.2a	–	–
1 month	0.2%	1.6 ± 0.0b	88.0 ± 2.0a	81.2 ± 3.6	N[2]
	0.5%	1.7 ± 0.1ab	91.0 ± 4.1a	89.0 ± 6.3	N
	1%	1.6 ± 0.1b	92.0 ± 2.9a	85.2 ± 6.1	N
2 months	0.2%	1.8 ± 0.1ab	93.0 ± 2.1a	93.0 ± 4.7	N
	0.5%	1.6 ± 0.1b	91.0 ± 3.1a	85.5 ± 5.9	N
	1%	1.7 ± 0.1ab	90.0 ± 2.6a	87.8 ± 6.1	N
3 months	0.2%	2.0 ± 0.1a	92.0 ± 2.5a	104.6 ± 5.8	P
	0.5%	1.8 ± 0.1ab	95.0 ± 2.2a	99.4 ± 6.5	N
	1%	1.8 ± 0.0ab	95.0 ± 1.7a	97.5 ± 2.8	N
P value					
Factor A		*	ns	–	–
Factor B		ns	ns	–	–
A × B		ns	ns	–	–

[1]Values are means ± SE of five replications for two trials (n = 10). Means were compared by Tukey's multiple comparison at 0.05 level. Similar lower-case letters in each column indicated that means are not significantly different.
[2]N = no phototoxicity, P = phytonutrient.
*Significant at the 0.05 level.

References

Abdel-Rahman, F.H., Alaniz, N.M. & Saleh, M.A. (2013). Nematicidal activity of terpenoids. *Journal of Environmental Science and Health, Part B* 48, 16-22. DOI: 10.1080/03601234.2012.716686

AccuWeather (2022). Current weather. Available online at https://www.accuweather.com/en/ke/nairobi/224758/current-weather/224758 (accessed 1 September 2022).

Barral, M.T. & Paradelo, R. (2011). A review on the use of phytotoxicity as a compost quality indicator. *Dynamic Soil, Dynamic Plant* 5, 36-44.

Beesa, N., Sasnarukkit, A., Jindapunnapat, K., Tivet, F., Bellafiore, S. & Chinnasri, B. (2021). Species characterization and population dynamics of *Hirschmanniella mucronata* in lowland rice fields managed under conservation agriculture in Cambodia. *Journal of the Saudi Society of Agricultural Sciences* 20, 137-145. DOI: 10.1016/j.jssas.2020.12.009

Beesa, N., Jindapunnapat, K., Chinnasri, B. & Chairin, T. (2022). Nematode development and changes in enzymatic defensive activity in rice plants upon *Meloidogyne graminicola* infection for preliminary screening of resistant cultivars. *Songklanakarin Journal of Science and Technology* 44, 26-31.

Birithia, R., Waceke, W., Lomo, P. & Masiga, D. (2012). Identification of root-knot nematode species occurring on tomatoes in Kenya: use of isozyme phenotypes and PCR-RFLP. *International Journal of Tropical Insect Science* 32, 78-84. DOI: 10.1017/S1742758412000173

Bowles, J., Blair, D. & McManus, D.P. (1992). Genetic variants within the genus *Echinococcus* identified by mitochondrial DNA sequencing. *Molecular and Biochemical Parasitology* 54, 165-174. DOI: 10.1016/0166-6851(92)90109-w

Brito, J.A., Kaur, R., Cetintas, R., Stanley, J.D., Mendes, M.L., Powers, T.O. & Dickson, D.W. (2010). *Meloidogyne* spp. infecting ornamental plants in Florida. *Nematropica* 40, 87-103.

CABI (2022). *Meloidogyne javanica* (sugarcane eelworm). Available online at https://www.cabi.org/isc/datasheet/33246 (accessed 1 September 2022).

Cameron, A.G. (2005). *Centrosema pascuorum* in Australia's Northern Territory: a tropical forage legume success story. *Tropical Grasslands* 39, 219.

Chen, J. & Song, B. (2021). Natural nematicidal active compounds: recent research progress and outlook. *Journal of Integrative Agriculture* 20, 2015-2031. DOI: 10.1016/S2095-3119(21)63617-1

Chin, S., Behm, C.A. & Mathesius, U. (2018). Functions of flavonoids in plant-nematode interactions. *Plants* 7, 85. DOI: 10.3390/plants7040085

Chitambo, O., Haukeland, S., Fiaboe, K.K.M. & Grundler, F.M.W. (2018). First report of *Meloidogyne hapla* and *M. javanica* coinfection on *Parthenium hysterophorus* in Kenya. *Plant Disease* 103, 162. DOI: 10.1094/PDIS-06-18-0964-PDN

Chitwood, D.J. (1992). Nematicidal compounds from plants. In: Nigg, H.N. & Seigier, D. (Eds). *Phytochemical resources for medicine and agriculture*. New York, USA, Plenum Press, pp. 185-204. https://link.springer.com/chapter/10.1007/978-1-4899-2584-8_8.

Clements, R.J., Winter, W.H. & Reid, R. (1984). Evaluation of some *Centrosema* species in small plots in Northern Australia. *Tropical Grasslands* 18, 83-91.

Coyne, D.L., Toko, M., Andrade, M., Hanna, R., Sitole, A., Kagoda, F., Andrade, M.I. & Marais, M. (2006). *Meloidogyne* spp. and associated galling and damage on cassava in Kenya and Mozambique. Available online at https://hdl.handle.net/10568/91821 (accessed 1 September 2022).

Coyne, D.L., Fourie, H.H. & Moens, M. (2009). Current and future management strategies in resource-poor regions. In: Perry, R.N., Moens, M. & Starr, J. (Eds). *Root-knot nematodes*. Wallingford, UK, CAB International, pp. 444-475.

Coyne, D.L., Cortada, L., Dalzell, J.J., Claudius-Cole, A.O., Haukeland, S., Luambano, N. & Talwana, H. (2018). Plant-parasitic nematodes and food security in Sub-Saharan Africa. *Annual Review of Phytopathology* 56, 381-403. DOI: 10.1146/annurev-phyto-080417-045833

Derycke, S., Vanaverbeke, J., Rigaux, A., Backeljau, T. & Moens, T. (2010). Exploring the use of cytochrome oxidase c subunit I (*COI*) for DNA barcoding of free-living marine nematodes. *PLoS ONE* 5, e13716. DOI: 10.1371/journal.pone.0013716

Desaeger, J. & Rao, M.R. (2000). Infection and damage potential of *Meloidogyne javanica* on *Sesbania sesban* in different soil types. *Nematology* 2, 169-178. DOI: 10.1163/156854100509060

Echeverrigaray, S., Zacaria, J. & Beltrão, R. (2010). Nematicidal activity of monoterpenoids against the root-knot nematode *Meloidogyne incognita*. *Phytopathology* 100, 199-203. DOI: 10.1094/PHYTO-100-2-0199

Garrido, M.S., Soares, A.C.F., Coimbra, J.L. & Sousa, C.S. (2008). Management of crotalaria and pigeon pea for control of yam nematode diseases. *Summa Phytopathologica* 34, 222-227. DOI: 10.1590/S0100-54052008000300003

Gichure, E. & Ondieki, J. (1984). Control of root knot nematode *Meloidogyne javanica* on potato *Solanum tuberosum* cultivar Annet in Kenya using 4 granular nematicides. *Pakistan Journal of Nematology* 2, 85-90.

Goodey, J.B., Franklin, M.T. & Hooper, D.J. (1965). *T. Goodey's 'The nematode parasites of plants catalogued under their hosts'*. Wallingford, UK, CABI Publishing.

Harender, K., Pankaj & Jagan, L. (2012). *Crotalaria juncea* for the management of root-knot nematode *Meloidogyne incognita* in tomato. *Indian Journal of Nematology* 42, 53-56.

Hartman, K. & Sasser, J. (1985). Identification of *Meloidogyne* species on the basis of differential host test and perineal-pattern morphology. In: Barker, K.R., Carter, C.C. & Sasser, J.N. (Eds). *An advanced treatise on* Meloidogyne*. Vol. II. Methodology*. Raleigh, NC, USA, North Carolina State University Graphics, pp. 69-77.

Holterman, M., van der Wurff, A., van den Elsen, S., van Megen, H., Bongers, T., Holovachov, O., Bakker, J. & Helder, J. (2006). Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. *Molecular Biology and Evolution* 23, 1792-1880. DOI: 10.1093/molbev/msl044

Hunt, D.J. & Handoo, Z.A. (2009). Taxonomy, identification and principal species. In: Perry, R.N., Moens, M. & Starr, J. (Eds). *Root-knot nematodes*. Wallingford, UK, CAB International, pp. 55-88. DOI: 10.1079/9781845934927.0055

Islam, M.T. & Faruq, A.N. (2008). Effect of selected soil amendments on seed germination, seedling growth and control of damping off of chilli seedlings. *Journal of Sher-e-Bangla Agricultural University* 2, 12-16.

Jindapunnapat, K., Reetz, N.D., MacDonald, M.H., Bhagavathy, G., Chinnasri, B., Soonthornchareonnon, N., Sasnarukkit, A., Chauhan, K.R., Chitwood, D.J. & Meyer, S.L.F. (2018). Activity of vetiver extracts and essential oil against *Meloidogyne incognita*. *Journal of Nematology* 50, 147-162. DOI: 10.21307/jofnem-2018-008

Jindapunnapat, K., Meyer, S.L.F., MacDonald, M.H., Reetz, N.D., Chitwood, D.J., Masler, E.P., Soonthornchareonnon, N., Camp, M.J., Sasnarukkit, A. & Chinnasri, B. (2019). Vegetable plant vigor and suppression of *Meloidogyne incognita* with vetiver shoot amendments in soil. *Nematropica* 49, 208-219.

Jones, J.T., Haegeman, A., Danchin, E.G.J., Gaur, H.S., Helder, J., Jones, M.G.K., Kikuchi, T., Manzanilla-López, R., Palomares-Rius, J.E., Wesemael, W.M.L. *et al.* (2013). Top 10 plant-parasitic nematodes in molecular plant pathology. *Molecular Plant Pathology* 14, 946-961. DOI: 10.1111/mpp.12057

Jourand, P., Rapior, S., Mateille, T. & Fargette, M. (2004). Nematostatic activity of aqueous extracts of West African *Crotalaria* species. *Nematology* 6, 765-771. DOI: 10.1163/1568541042843513

Kankam, F., Suen, F.A. & Adomako, J. (2015). Nematicidal effect of sunn hemp *Crotalaria juncea* leaf residues on *Meloidogyne incognita* attacking tomato *Solanum lycopersicum* roots. *Journal of Crop Protection* 4, 241-246.

Kanyagia, S.T. (1993). *Nematodes attacking the purple passion fruit* (Passiflora edulls) *plants in Kenya and studies on the biology of two common species*, Meloidogyne javanica *and* M. incognita *with special reference to their pathogenicity and chemical control*. Ph.D. Thesis, University of Nairobi, Nairobi, Kenya.

Kegode, T.M., Bargul, J.L., Mokaya, H.O. & Lattorff, H.M.G. (2022). Phytochemical composition and bio-functional properties of *Apis mellifera* propolis from Kenya. *Royal Society Open Science* 9, 211214. DOI: 10.1098/rsos.211214

Kiriga, A.W., Coyne, D., Atandi, J.G., Beek, N.V. & Haukeland, S. (2021). Diversity and population densities of plant-

parasitic nematodes in commercial and smallholder pineapple production in Kenya. *Nematology* 23, 837-850. DOI: 10.1163/15685411-bja10080

Lopes-Caitar, V.S., Pinheiro, J.B. & Marcelino-Guimarães, F.C. (2019). Nematodes in horticulture: an overview. *Journal of Horticultural Science and Crop Research* 1, 105.

Maina, K.I. (2016). *Identification and management of root knot nematodes* (Meloidogyne *spp.*) *attacking chickpea* (Cicer arietinum *L.*) *in Nakuru County, Kenya using nematicides and cultural practices*. M.Sc. Thesis, Egerton University, Nairobi, Kenya.

Malik, S.K., Ahmad, M. & Khan, F. (2017). Qualitative and quantitative estimation of terpenoid contents in some important plants of Punjab, Pakistan. *Pakistan Journal of Science* 69, 150.

Masson, A., Vermeire, M., Leng, V., Simonin, M., Tivet, F., Thi, H.N., Brunel, C., Suong, M., Kuok, F., Moulin, L. *et al.* (2022). Enrichment in biodiversity and maturation of the soil food web under conservation agriculture is associated with suppression of rice-parasitic nematodes. *Agriculture, Ecosystems & Environment* 331, 107913. DOI: 10.1016/j.agee.2022.107913

Meyer, S., MacDonald, M.H., Reetz, N.D., Kantor, M.R., Carta, L., Handoo, Z.A., Camp, M.J. & Phillips, T.D. (2020). Chia: host status for *Meloidogyne incognita* and activity of plant extracts. *Plant Disease* 104, 2979-2985. DOI: 10.1094/PDIS-10-19-2171-RE

Meyer, S.L.F., Zasada, I.A., Roberts, D.P., Vinyard, B.T., Lakshman, D.K., Lee, J., Chitwood, D.J. & Carta, L.K. (2006). *Plantago lanceolata* and *Plantago rugelii* extracts are toxic to *Meloidogyne incognita* but not to certain microbes. *Journal of Nematology* 38, 333-338.

Moens, M., Perry, R.N. & Starr, J.L. (2009). *Meloidogyne* species – a diverse group of novel and important plant parasites. In: Perry, R.N., Moens, M. & Starr, J.L. (Eds). *Root-knot nematodes*. Wallingford, UK, CAB International, pp. 1-17.

Moldes, A.B., Vázquez, M., Domínguez, J.M., Diaz-Fierros, F. & Barral, M.T. (2007). Evaluation of mesophilic biodegraded grape marc as soil fertilizer. *Applied Biochemistry and Biotechnology* 141, 27-36. DOI: 10.1007/s12010-007-9208-2

Moosavi, M.R. (2015). Damage of the root-knot nematode *Meloidogyne javanica* to bell pepper, *Capsicum annuum*. *Journal of Plant Diseases and Protection* 122, 244-249. DOI: 10.1007/BF03356559

Mwangi, M.W., Kimenju, J.W., Narla, R.D., Muiru, W.M. & Kariuki, G.M. (2017). Evaluation of selected tomato cultivars reaction to infestation with *Meloidogyne javanica* in greenhouse conditions. *International Journal of Agronomy and Agricultural Research* 11, 17-25.

Ngundo, B.W. & Taylor, D.P. (1974). Effects of *Meloidogyne* spp. on bean yields in Kenya. *Plant Disease Reporter* 58, 1020-1023.

Njuguna, L.K. & Bridge, J. (1998). Plant-parasitic nematodes of Irish potatoes (*Solanum tuberosum*) in Central Province and sweet potatoes (*Ipomoea batatas*) in Central, Nyanza and Coast Provinces of Kenya. *International Journal of Nematology* 8, 2-26.

Nunn, G.B. (1992). *Nematode molecular evolution*. Ph.D. Thesis, University of Nottingham, Nottingham, UK.

Ohri, P. & Pannu, S.K. (2010). Effect of phenolic compounds on nematodes – a review. *Journal of Applied and Natural Science* 2, 344-350. DOI: 10.31018/jans.v2i2.144

Oliveira, D.F., Costa, V.A., Terra, W.C., Campos, V.P., Paula, P.M. & Martins, S.J. (2019). Impact of phenolic compounds on *Meloidogyne incognita in vitro* and in tomato plants. *Experimental Parasitology* 199, 17-23. DOI: 10.1016/j.exppara.2019.02.009

Osei, K., Gowen, S.R., Pembroke, B., Brandenburg, R.L. & Jordan, D.L. (2010). Potential of leguminous cover crops in management of a mixed population of root-knot nematodes (*Meloidogyne* spp.). *Journal of Nematology* 42, 173-178.

Parlevliet, J.E. (1971). Root-knot nematodes, their influence on the yield components of pyrethrum and their control. *Acta Horticulturae* 21, 201-206. DOI: 10.17660/ActaHortic.1971.21.34

Patel, S. & Dhillon, N.K. (2017). Evaluation of sunnhemp (*Crotalaria juncea*) as green manure /amendment and its biomass content on root knot nematode (*Meloidogyne incognita*) in successive crop brinjal. *Journal of Entomology and Zoology Studies* 5, 716-720.

Philbrick, A.N., Adhikari, T.B., Louws, F.J. & Gorny, A.M. (2020). *Meloidogyne enterolobii*, a major threat to tomato production: current status and future prospects for its management. *Frontiers in Plant Science* 11, 606395. DOI: 10.3389/fpls.2020.606395

Popova, M., Silici, S., Kaftanoglu, O. & Bankova, V. (2005). Antibacterial activity of Turkish propolis and its qualitative and quantitative chemical composition. *Phytomedicine* 12, 221-228. DOI: 10.1016/j.phymed.2003.09.007

Rocha, T.L., Soll, C.B., Boughton, B.A., Silva, T.S., Oldach, K., Firmino, A.A.P., Callahan, D.L., Sheedy, J., Silveira, E.R., Carneiro, R.M.D.G. *et al.* (2017). Prospection and identification of nematotoxic compounds from *Canavalia ensiformis* seeds effective in the control of the root knot nematode *Meloidogyne incognita*. *Biotechnology Research and Innovation* 1, 87-100. DOI: 10.1016/j.biori.2017.10.003

Rusinque, L., Nóbrega, F., Cordeiro, L., Lima, A., Andrade, S. & Inácio, M.L. (2022). Root-knot nematode species associated with horticultural crops in the island of Azores, Portugal. *Horticulturae* 8, 101. DOI: 10.3390/horticulturae8020101

Sharma, S.B., Siddiqi, M.R., Omanp, P. & Singh, L. (1993). Distribution and potential importance of plant-parasitic nematodes associated with pigeon pea in Kenya. *Afro-Asian Journal of Nematology* 3, 182-187.

Suong, M., Chapuis, E., Leng, V., Tivet, F., Waele, D., Thi, H.N. & Bellafiore, S. (2019). Impact of a conservation agriculture

system on soil characteristics, rice yield, and root-parasitic nematodes in a Cambodian lowland rice field. *Journal of Nematology* 51, 1-15. DOI: 10.21307/jofnem-2019-085

Talavera-Rubia, M., Vela-Delgado, M.D. & Verdejo-Lucas, S. (2020). Nematicidal efficacy of milbemectin against root-knot nematodes. *Plants* 9, 839. DOI: 10.3390/plants9070839

Tuncsoy, B. (2021). Nematicidal activity of silver nanomaterials against plant-parasitic nematodes. In: Abd-Elsalam, K.A. (Ed.). *Silver nanomaterials for agri-food applications*. Amsterdam, The Netherlands, Elsevier, pp. 527-548.

Viaene, C.N., Coyne, D.L. & Kerry, B.R. (2006). Biological and cultural management. In: Perry, R.N. & Moens, M. (Eds). *Plant nematology*. Wallingford, UK, CAB International, pp. 346-369.

Wang, K.H., Sipes, B.S. & Schmitt, D.P. (2002). *Crotalaria* as a cover crop for nematode management: a review. *Nematropica* 32, 35-57.

Wendimu, G.Y. (2021). Biology, taxonomy, and management of the root-knot nematode (*Meloidogyne incognita*) in sweet potato. *Advances in Agriculture* 2021, 8820211. DOI: 10.1155/2021/8820211

Wuyts, N., Swennen, R. & De Waele, D. (2006). Effects of plant phenylpropanoid pathway products and selected terpenoids and alkaloids on the behaviour of the plant-parasitic nematodes *Radopholus similis*, *Pratylenchus penetrans* and *Meloidogyne incognita*. *Nematology* 8, 89-101. DOI: 10.1163/156854106776179953

Yang, X., Wang, X., Wang, K., Su, L., Li, H., Li, R. & Shen, Q. (2015). The nematicidal effect of *Camellia* seed cake on root-knot nematode *Meloidogyne javanica* of banana. *PLoS ONE* 10, e0119700. DOI: 10.1371/journal.pone.0119700

Zijlstra, C., Donkers-Venne, D.T.H.M. & Fargette, M. (2000). Identification of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* using sequence characterised amplified region (SCAR) based PCR assays. *Nematology* 2, 847-853. DOI: 10.1163/156854100750112798