GROUND WILD CUCUMBER FRUITS SUPPRESS NUMBERS OF *MELOIDOGYNE INCOGNITA* ON TOMATO IN MICROPLOTS

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ABSTRACT

Mashela, P. W. 2002. Ground wild cucumber fruits suppress numbers of *Meloidogyne incognita* on tomato in microplots. Nematropica 32:13-19.

Ground wild cucumber (*Cucumis myriocarpus*) fruits were evaluated as a soil amendment in microplots infested with the root-knot nematode, *Meloidogyne incognita*. Effects of the amendment on nematode numbers, growth of tomato (*Lycopersicon esculentum*), soil electrical conductivity, and soil pH were evaluated during spring 1999 and again during fall 2000. Treatments included an untreated control, *M. incognita*-infested soil, *C. myriocarpus*-amended soil, and *C. myriocarpus*-amended + *M. incognita*-infested soil, arranged in a randomized complete block design with 15 replicates. Seedlings were inoculated with nematodes and treated with amendment (0.71 mt/ha) one day after transplanting and plants were harvested 83 days later. Relative to nematodes alone, *C. myriocarpus*-amended + *M. incognita*-infested soil had 73-83% and 49-68% fewer nematode numbers in roots and soil, respectively. This treatment increased plant height, dry matter weight, fresh fruit weight and soil electrical conductivity by 13-34%, 76-135%, 226-538% and 36-38%, respectively. Nematodes alone reduced soil pH, whereas *C. myriocarpus* amendment did not affect soil pH nor leaf nutrient elements. Amendment with *C. myriocarpus* increased soil electrical conductivity in both spring and fall experiments. Results indicated that *C. myriocarpus* fruits have potential as an organic amendment for managing *M. incognita* in tomato production.

Key words: Cucumis myriocarpus, electrical conductivity, *Meloidogyne incognita,* nematode, organic amendment, root-knot nematode, pH, wild cucumber.

RESUMEN

Mashela, P. W. 2002. Frutos molidos de pepino silvestre reducen densidades de *Meloidogyne incognita* en tomate cultivado en microparcelas. Nematrópica 32:13-19.

Frutos molidos de pepino silvestre (*Cucumis myriocarpus*) se evaluaron como emiendas de suelo en microparcelas. Se analizó su efecto sobre el nematodo nodulador (*Meloidogyne incognita*), crecimiento del tomate (*Lycopersicon esculentum*), conductividad eléctrica del suelo y pH durante la primavera de 1999 y el otoño 2000. Como tratamientos se incluyó suelos no infectados (control), suelos infectados con *M. incognita*, aplicación de *C. myriocarpus* como emienda, y aplicación de *C. myriocarpus* en suelos infectados con *M. incognita*. Se utilizó un diseño de bloques completamente al azar con 15 repeticiones. Un día después del transplante, las plantas fueron inoculadas con juveniles del segundo estado, 7,100 (primavera) y 6,600 (otoño). Frutos molidos de *C. myriocarpus* se aplicaron en dosis de 0.71 TM/ha en el día de la inoculación. Las plantas se cosecharon a los 83 días después de aplicados los tratamientos. En relación a la presencia de nematodos, *C. myriocarpus* + *M. incognita* redujo el número de nematodos en raíces y suelo en 73-83% y 49-68%, respectivamente. Este tratamiento incrementó altura de planta, peso de materia seca, peso fresco de frutos y conductividad eléctrica del suelo en valores de 13-34%, 76-135%, 226-538% y 36-38%, respectivamente. La sola presencia de nematodos redujo el pH del suelo, mientras que *C. myriocarpus* no afectó el pH, y tampoco el contenido de minerales en el follaje. Emiendas con *C. myriocarpus* incrementaron la conductividad eléctrica del suelo en los experimentos de la primavera y el otoño. Los resultados indican que frutos de *C. myriocarpus* tienen potencial como enmiendas orgánicas en el manejo de *M. incognita* en la producción de tomate.

Palbras claves: Cucumis myriocarpus, conductividad eléctrica, *Meloidogyne incognita,* nematodo, emienda orgánica, nematodo nodulador de la raíz, pH, pepino silvestre.

INTRODUCTION

The literature concerning suppression of plant-parasitic nematode densities by organic amendments is replete with both promising and inconsistent results (Mankau and Minteer, 1962; Mankau, 1968; Muller and Gooch, 1982; Rodriguez-Kabana, 1986; Stirling, 1991; McSorley and Gallaher, 1995a,b). Generally, large quantities of organic amendments are required for the effective suppression of nematode densities (Rodriguez-Kabana, 1986; Stirling, 1991; McSorley and Gallaher, 1995a). Efficacy on nematode suppression by these materials depends on the C:N ratio and the state of decomposition (Stirling, 1991). Properly decomposed materials release mineral elements into soil solution, increasing osmotic potential of soil solutions (Bohn *et al.*, 1985; Stirling, 1991). However, low osmotic potential had been associated with the reduction of densities of various nematode species (Dropkin *et al.*, 1958; Kirkpatrick and Van Gundy, 1966; Mashela *et al.*, 1992); therefore, other mechanisms are likely involved in suppression of nematode numbers by organic amendments. McSorley and Gallaher (1995a) noted that research on quality and quantity of amendments was needed to enhance widespread use of these materials.

Certain plant organs contain concentrated toxic chemical compounds that are lethal to animals in small quantities (Cooper Driver, 1983). In ground form, these plant organs, referred to as "mutis," are widely used as traditional medicines for human beings and livestock in the Republic of South Africa (Van Wyk *et al.*, 1997). Recently, Mashela and Mphosi (2001) developed an alternative organic amendment technology using mutis, by which nematode suppression was consistently attained using small quantities of organic amendment. When applied at 0.71 mt/ha, ground wild cucumber (*Cucumis myriocarpus* Naud.) fruits suppressed root and soil stages of *Meloidogyne incognita* (Kofoid & White) Chitwood on greenhouse tomato by 92-98%. The material increased soil electrical conductivity (EC) but had no effect on soil pH. Ground leaves of another widely used "muti", fever tea (*Lippia javanica* L.), had similar effects on nematodes and soil EC and also reduced soil pH (Mashela and Ngobeni, 2001). However, these amendments have not been tested under field conditions. The objective of this study was to evaluate effects of ground *C. myriocarpus* fruits on densities of *M. incognita*, growth of tomato (*Lycopersicon esculentum* Mill.) plants, soil EC and soil pH in microplots in the field.

MATERIALS AND METHODS

The experiment was conducted during spring 1999 and repeated during fall 2000 on different sites at the Horticultural Skills Centre, University of the North (23°53'10"S, 29°44'15"E). *Cucumis myriocarpus* fruits were collected from Zebediela district (24°28'S, 24°28'E) during winter 1999, chopped into pieces and dried for 5 days at 52°C in forced-air ovens prior to grinding in a Wiley mill through a 1-mm sieve. *Cucumis myriocarpus* fruits contained 31.5 g Ca, 6.3 g S, 22.1 g Cl, 29.6 g Mg, 28.9 g K, 44.2 g P, 6.7 g Na, 35 mg Cu, 1,811 mg Fe, 37 mg Mn, 3 mg Mo, 93 mg Zn and 3,860 g C and 279 g N/kg dry matter, for a C:N ratio of 14:1.

Plots comprised 30-cm-diam plastic pots, inserted into holes dug at 0.6 m × 0.5 m and filled with 2.8 liters steam-pasteurized Hutton soil collected from the topsoil at the trial site. The soil consisted of 64% sand, 32% clay, 4% silt, 1.6% organic C, EC 0.165 dS/m and pH-H_2O 6.63. Prior to transplanting, plots were drench-fertilized with 2.5 g of 2:3:2 N:P:K (22% active) + 0.5% Zn and 1.0 g of 2:1: 2 N:P:K (43% active) that provided a total of 0.35-mg N, 0.32-mg K and 0.32-mg P per plant. The latter fertilizer also provided 0.9-mg Mg, 0.75-mg Fe, 0.075 mg Cu, 0.35-mg Zn, 1.0-mg B, 3.0-mg Mn and 0.07-mg Mo/ml water. A day after irrigating the plots to field capacity, uniform 2-week-old 'Floradade' tomato seedlings were transplanted.

Four treatments were included in the tests: an untreated control, soil amendment with ground *C. myriocarpus*, soil infested with *M. incognita* Race 1, and soil amended with *C. myriocarpus* + *M. incognita*. Both experiments were arranged in a randomized complete block design with 15 replications of each treatment. Nematode inoculum was prepared by extracting eggs from roots of greenhouse-grown tomato ('Floradade') with 1% NaOCl (Hussey and Barker, 1973) and collecting juveniles (J2) after the eggs had been incubated for four days on modified Baermann trays (Rodriguez-Kabana and Pope, 1981). Appropriate pots were infested with 7,100 (spring) or 6,600 (fall) J2 in 10-cm deep holes at the base of each plant. *Cucumis myriocarpus* fruit material were applied at 5 g/pot (0.71 mt/ha) on the soil surface around the seedlings immediately following addition of the nematodes.

Two tensiometers were inserted at 20- and 30-cm depths in two arbitrarily selected pots of each treatment, and pots were irrigated with 1,000 cm^3 tap water when tensiometer readings averaged 15-20 kPa. Spring and fall ambient day/night temperatures averaged 28/20 and 26/17°C, respectively.

At harvest, 83 days after the treatments, plant height and fresh fruit weight were recorded. Soil samples for nematode, EC and pH analyses were collected using a 2.5-cm-diam. auger. Each sample consisted of 10 soil cores collected at 20-cm depths. Small roots were removed from cores and returned to their respective pots and composited cores were thoroughly mixed. A 100-cm^3-subsample was removed for nematode extraction using a modified sieving-and-centrifugation method (Jenkins, 1964). The remaining soil samples were air-dried and used to determine soil EC and soil pH. Root systems were removed and immersed in water to remove soil particles. Nematodes were extracted from 5 g roots/plant by macerating and blending roots for 30 s in 1% NaOCl (Hussey and Barker, 1973), followed by rinsing through nested sieves (150-µm, 45-µm, and 25-µm openings). Material collected from the 25-µm sieve was extracted for six days using a modified Baermann method (Rodriguez-Kabana and Pope, 1981). Second stage juveniles were counted with a compound microscope. Combining the two extraction methods excluded confounding eggs of plant-parasitic nematodes for those of free-living nematodes, invariably inflating nematode counts (McSorley and Gallaher, 1995a). Shoots were oven-dried for 72 hours at 70°C, weighed, and then leaves were ground and analyzed for nutrient elements (Rhue and Kidder, 1983).

Statistical analyses were conducted using SAS software (SAS Institute, Inc., Cary, NC, U.S.A.). Data for J2 were analyzed using a two-sample t-test, whereas plant and soil data were subjected to analysis of variance (ANOVA). Mean separation was accomplished using Duncan's multiple-range test when F-values were significant at $P \leq 0.05$.

RESULTS

Compared to nematodes alone, nematode densities in *C. myriocarpus* + *M. incognita* were lower during both seasons (Table 1). In spring, relative to nematode alone, *C. myriocarpus* amendment reduced densities of *M. incognita* in soil by 49% and in roots by 83%. When the experiment was repeated in the fall, nematode densities in soil and roots were reduced by 68% and 73%, respectively, by treatment with *C. myriocarpus*.

The nematode treatment reduced tomato plant height, dry shoot weight, and fresh fruit weight relative to the untreated control (Table 2). Amendment of nematode-free plots with *C. myriocarpus* increased these parameters, but did not result in yield, plant weight, or height as great as the untreated control. Relative to nematodes alone, *C. myriocarpus* added to nematode-treated plots increased plant weight, plant height and fruit weight in spring by 338%, 34% and 538%, respectively. In fall, the relative increases for these parameters were 135%, 13% and 226%, respectively. Phytotoxicity was not observed. Treatments had no effect on leaf nutrient elements, except that Fe concentrations were highest in plant tissues of treatments amended with *C. myriocarpus* (data not shown).

Cucumis myriocarpus alone or in combination with nematodes had no effect on soil pH, whereas nematodes alone reduced soil pH (Table 3). Relative to untreated control, nematode alone lowered soil pH by 3% in spring and 4% in fall. Regardless of the presence or absence of nematodes, amendment with *C. myriocarpus* increased soil EC. Relative to untreated control, treatment with *C. myriocarpus* increased soil EC by 68% in spring and 50% in fall, whereas *C. myriocarpus* + *M. incognita* increased soil EC by 79% in spring and 65% in fall.

DISCUSSION

A reduction in the numbers of *M. incognita* due to *C. myriocarpus* amendment during both seasons was consistent with previous greenhouse observations (Mashela and Mphosi, 2001). The effective application rate of 0.71 mt/ha is far lower than that generally required (10-100 mt/ha) to achieve nematode suppression with organic ammendments (McSorley and Gallaher, 1995a; Rodriguez-Kabana, 1986; Stirling, 1991). These results are promising, because high application rates are a major constraint to the use of organic amendments for nematode management.

Table 1. Effects of *Cucumis myriocarpus* organic amendment on juvenile densities of *Meloidogyne incognita* on tomato.

	Spring 1999		Fall 2000	
Treatment	Juveniles (100 cm^3 soil)	Juveniles (g fresh roots)	Juveniles (100 cm^3 soil)	Juveniles (g fresh roots)
Nematode	182	150	138	139
Cucumis + nematode	93	25	44	38
% reduction[z]	49**	83**	68**	73**

[z]Treatment effect = [1.0 - (*Cucumis* + nematode/nematode)] × 100. Asterisks denote significance at P = 0.01.

Table 2. Effect of *Cucumis myriocarpus* organic amendment and *Meloidogyne incognita* on growth of tomato plants and fruit yield.

Season	Treatment	Shoot weight (g)	Plant height (cm)	Fruit yield (g)
Spring 1999	Control	38.05 a[z]	76.15 a	566.91 a
	Cucumis	39.53 a	73.91 a	575.65 a
	Nematode	6.70 c	49.50 c	56.04 c
	Cucumis + nematode	29.35 b	66.11 b	357.36 b
Fall 2000	Control	43.15 a	80.48 a	616.88 a
	Cucumis	46.22 a	79.09 a	622.67 a
	Nematode	11.37 c	56.15 c	99.10 c
	Cucumis + nematode	26.73 b	63.52 b	322.70 b

[z]Column means (n = 15) followed by the same letter were not different ($P = 0.05$) according to Duncan's multiple-range test.

The component of *C. myriocarpus* fruits responsible for suppression of *M. incognita* is unknown. However, it has been established that fruits and roots of *C. myriocarpus* contain large quantities of highly toxic cucumin ($C_{27}H_{40}O_9$) and leptodermin ($C_{27}H_{38}O_8$), collectively referred to as cucurbitacins (Van Wyk *et al.*, 1997). The water-soluble cucurbitacins, with strong cutaneous adsorptive properties, are amongst the bitterest known substances.

Under conventional soil amendment systems, decomposition of organic materials is a prerequisite for suppression of nematodes and the efficacy of materials depends upon the C:N ratio (Stirling, 1991; McSorley and Gallaher, 1995a,b; Ritzinger and McSorley, 1997). Organic materials with C:N ratio less than 20:1 have higher degradation rates and often nematicidal activities (McSorley and Gallaher, 1995a,b). High decomposition rates have

Table 3. Effect of *Cucumis myriocarpus* organic amendment and *Meloidogyne incognita* on soil pH and soil electrical conductivity.

	Spring 1999		Fall 2000	
Treatment	pH	EC (dS/m)	pH	EC (dS/m)
Control	6.79 a[z]	0.163 b	6.74 a	0.175 b
Cucumis	6.73 a	0.274 a	6.69 a	0.263 a
Nematode	6.58 b	0.183 b	6.45 b	0.178 b
Cucumis + nematode	6.69 a	0.291 a	6.68 a	0.189 a

[z]Column means (n=15) followed by the same letter were not different ($P = 0.05$) according to Duncan's multiple-range test.

been associated with increased numbers of nematode antagonists and the release of nutrient elements (Muller and Gooch, 1982; Stirling, 1991). The C:N ratio (14:1) of *C. myriocarpus* fruits is within the recommended range to achieve these effects. However, because *C. myriocarpus* was applied at a relatively low rate and in an undecomposed state at the time of nematode treatment, the events involved in degradation may be of little importance in the efficacy of this organic amendment. Due to the known chemical composition of *C. myriocarpus*, it appears likely that potent nematicidal or nematistatic chemicals were leached from the *C. myriocarpus* preparation. Grinding of the dried fruit may be important to this process because it increases the surface area of the organic amendment.

In addition to increased microbial activity, and suppression of nematodes or other pathogens, improvement in crop yields due to conventional organic amendments can also occur through release of mineral nutrients (Muller and Gooch, 1982; Stirling, 1991). The improvement in plant growth of tomato in *C. myriocarpus*-amended soil with nematodes, compared to the nematode alone did not appear to be a nutritional response. Plant growth and yield did not differ between the untreated control and the *C. myriocarpus* treatment, demonstrating a lack of phytotoxicity and a lack of fertilizer effect in tomato at the applied rates. Moreover, nutrient content in tomato leaves suggested that the release of mineral nutrients (except possibly Fe) by *C. myriocarpus* was negligible. Because *C. myriocarpus* had no direct effect on plant growth or the accumulation of nutrient elements in plant tissues, the observed growth increase of nematode-infected plants in response to *C. myriocarpus* amendment was likely due primarily to the suppression of nematodes.

Most toxic chemical compounds in plants are produced as secondary metabolites that originate as organic acids (Rice, 1984), with the potential for reducing soil pH and subsequent changes in the availability of mineral nutrients (Bohn *et al.*, 1985). *Cucumis myriocarpus* amendment had no effect on soil pH, which enhances the potential use of this material. Because pH for untreated control and *C. myriocarpus* + *M. incognita* treatments did not differ, the reduction in soil pH under nematode alone was possibly due to plant responses to high nematode infection levels. Root-knot nematode syncytia have increased concentrations of amino acids (Wallace, 1973). Under high nematode numbers the amino acids and other related acids are possibly released into the rhizosphere through root exudation.

Increased soil EC in this and related studies (Mashela and Mphosi, 2001; Mashela and Nthangeni, 2001; Mashela and Ngobeni, 2002) supported the view that organic amendments released nutrient elements into soil solution (Stirling, 1991). However, as noted previously, the release of essential nutrient elements was negligible, possibly due to the small quantities applied. It is also possible that the released nematicidal compounds are responsible for elevated EC.

Highly toxic fruits of *C. myriocarpus* have potential as an organic amendment for managing densities of *M. incognita* in tomato production in rural poor communities of the Northern Province in the Republic of South Africa. In these communities, vegetative materials of *C. myriocarpus* serve as a local food, whereas the widely available fruits are used in various traditional medicines (Van Wyk *et al.*, 1997). The introduction of *C. myriocarpus* as an organic amendment for nematode suppression may have a considerable application for subsistence farming systems in Africa and other regions.

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