Crop Protection 40 (2012) 69–72

Contents lists available at SciVerse ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Interactive effects of *Meloidogyne incognita* race 2, *Bradyrhizobium japonicum* and crude extracts of *Cucumis myriocarpus* fruit on *Vigna unguiculata*

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ARTICLE INFO

Article history:
Received 5 August 2011
Received in revised form
2 February 2012
Accepted 16 April 2012

Keywords:
Bradyrhizobium japonicum
Cowpea
Cucumis myriocarpus
Meloidogyne incognita
Nodules

ABSTRACT

Crude extracts of wild cucumber (*Cucumis myriocarpus*) fruit, without information on their effects on nodulation, had been touted as having potential as a nematicide and a fertiliser in low-input cowpea (*Vigna unguiculata*) production systems. Interactive effects of the southern root-knot nematode (*Meloidogyne incognita*), *Bradyrhizobium japonicum* and crude extracts of wild cucumber (*C. myriocarpus*) fruit on each other and growth of cowpea variety Eureka were investigated in a 23 factorial experiment. At harvest, 70 days after initiating the treatments, significant ($P \leq 0.05$) first order interactions between any two of the three main factors on variables measured were observed, with interactive effects being either stimulatory or inhibitory. Crude extracts of *C. myriocarpus* fruit were basically suppressive on nematode numbers but stimulated nodulation and growth of cowpea. Consequently, the material is suitable for use in managing population densities of *M. incognita* on cowpea production in low-input cowpea production systems.

1. Introduction

Farmers have been arbitrarily classified as household, subsistence, emerging and commercial farmers in Limpopo Province, South Africa, in order to tailor-make and fast-track farmer-support services (Anon., 2009). Household and subsistence farmers had been reliant on low-input agricultural systems. Organic matter and animal manure invariably play a major role in improving crop yield, with cropping systems comprising a monoculture of an intercropped system which includes maize (*Zea mays*), cowpea (*Vigna unguiculata*), watermelon (*Citrullus lanatus*) and other minor crops. Due to the traditional "monocultural system" which is more cultural than agricultural, the build-up of root-knot nematodes (*Meloidogyne* species) in these farming systems have since reached population levels that, in some cases, resulted in total crop failure among the constituents of the intercropping system (Fourie and Mc Donald, 2003). Cowpea is included in the farming systems primarily for green leaves and immature pods, which are highly nutritious (Ayisi et al., 2000), whereas grains are retained for propagation in the next season. Generally, due to repeated harvests of greens and immature pods, grain yield constitutes a negligible portion of the yield components.

A ground-leaching technology (GLT) system was originally developed to ameliorate drawbacks of organic amendments in management of plant-parasitic nematodes in low-input farming systems in Limpopo Province (Mashela, 2002). In GLT system, 0.20–0.80 t/ha crude extracts of wild cucumber (*Cucumis myriocarpus*) fruit are applied at 2–5 g material/plant by placing the material in a shallow hole around the stem soon after transplanting (Mashela, 2002). The technology drastically reduced quantities of organic material required to effect nematode suppression, transport costs, availability challenges, negative period, inconsistency of reducing nematode numbers and alteration of soil pH. Generally, in conventional organic amendments, large quantities of organic material (10–250 t/ha) are required to effect nematode suppression, which translates to high transport costs and availability of materials, a waiting period to allow for microbial degradation and thus, avoidance of negative period, with nematode suppression results that are highly inconsistent (Mashela, 2002; Mashela and Nthangeni, 2002; Stirling, 1991). Currently, the GLT system is widely used in low-input agricultural systems in Limpopo Province, with consistent results in nematode suppression and has fertiliser effect on plant growth (Mashela, 2002; Mashela and Mpati, 2002; Mashela and Mphosi, 2002; Mashela and Nthangeni, 2002; Mashela et al., 2010). Also, the efficacy of crude extracts of *C. myriocarpus* fruit in suppression of nematodes and improving plant growth were comparable to those of synthetic nematicides, *viz*. aldicarb and fenamiphos (Mashela et al., 2008).

In household and subsistence farming systems, cowpea (*V. unguiculata*) forms an integral part of the intercrops (Ayisi et al., 2000), and serves an important role in alleviating food insecurity in

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0261-2194/$ – see front matter © 2012 Published by Elsevier Ltd.
doi:10.1016/j.cropro.2012.04.017

marginal communities. The performance of this crop is dependent on the efficacy of *rhizobium* species, which assimilate elemental nitrogen to nitrates for use by plants in exchange for carbohydrates (Ayisi et al., 2000). Extrinsic physical factors like synthetic nematicides, fertilisers, herbicides and organic matter have had negative effects on nodulation, and therefore, growth of cowpea (Ayisi et al., 2000). Inhibitory effect of crude extracts of *C. myriocarpus* fruit on nodulation would render this material unacceptable to cowpea-producing farmers. Interactive effects of *Meloidogyne incognita*, *Bradyrhizobium japonicum* and crude extracts of *C. myriocarpus* fruit on final nematode population density (Pf), nodulation and growth of cowpea are not documented. Therefore, the objective of our study was to investigate the interactive effects of *M. incognita*, *B. japonicum* and crude extracts of *C. myriocarpus* fruit on Pf, nodulation and growth of cowpea.

2. Materials and methods

2.1. Preparation of experimental units and material

A microplot experiment was conducted during summer (November–January) in 2009/2010 and repeated in 2010/2011 at the Plant Protection Skills Centre, University of Limpopo (UL), South Africa (23°53′10″S, 29°44′15″E). The location had Hutton sandy loam (65% sand, 30% clay, 5% silt) containing 1.6% organic C, with EC = 0.148 dS m^{-1}, pH(H_2O) = 6.5 and hot dry summers, with day maximum temperature (DMT) ranges of 28–38 °C. Average annual rainfall at the location is 500 mm.

Microplots were established by inserting 30-cm-diameter plastic pots into 20-cm-deep holes at 1.0 m intra-row and 0.5 m inter-row spacing. Seedlings of cowpea variety Eureka were raised in 200-hole seedling trays containing Hygromix (Hygrotech, Pretoria North, South Africa). Uniform 2-week old seedlings were transplanted into pots each filled with 10 L steam-pasteurised soil collected from the topsoil of dug holes used to insert pots. Fruit of *C. myriocarpus* were raised and at maturity (Mafeo and Mashela, 2009), fruit were collected, cut into pieces and prepared to serve as crude extracts (Mashela, 2002).

2.2. Experimental design

Eight factorial treatments (2 × 2 × 2), *viz.* untreated control ($M_oB_oC_o$), *M. incognita* race 2 ($M_1R_oC_o$), *B. japonicum* ($M_oB_1C_o$), *C. myriocarpus* ($M_oB_oC_1$), $M_1B_oC_1$, $M_1B_1C_o$, $M_oB_1C_1$ and $M_1B_1C_1$, were arranged in a randomised complete block design, with 10 replicates. A day after transplanting, each cowpea seedling was inoculated by dispensing ca. 1000 *M. incognita* race 2 eggs and second-stage juveniles (J2s), which were prepared by extracting from roots of greenhouse-grown nematode-susceptible kenaf (*Hibiscus cannabinus*) plants in 1% sodium hyperchlorite (NaOCl) solution in water (Hussey and Barker, 1973). A 20-ml plastic syringe was used to place eggs and J2s into 5-cm-deep holes on the cardinal points of the stem, whereas plants without nematodes each received a 20 ml filtrate from the nematode aliquot in order to establish microbes associated with nematodes. Relevant pots were amended with 3 g crude extracts of *C. myriocarpus* in separate holes from which nematodes were placed.

2.3. Cultural practices

Irrigation was scheduled using four Hadeco Moisture Meters (Hadeco Magic^R, New Delhi, India) with plants being irrigated when the average reading was less than 2 units. A day before transplanting and thereafter, once monthly, each pot was irrigated with 2 L half-strength Hoagland solution without N (Hoagland and Arnon, 1950). Plants were monitored for aphids and sprayed with Malathion as per label instruction whenever at least 5 aphids per plant were seen. Weeds among pots were removed using hand-held hoes when necessary. Total rainfall in 2009/2010 and 2010/2011 during the experiments was 31 mm and 53 mm, respectively.

2.4. Data collection

At harvest, 70 days after transplanting, plant height was measured from the crown to the terminal end of the flag leaf, stems were cut at the crown and stem diameters measured at 5 cm above the severed ends using a digital vernier caliper. Shoots were oven-dried at 70 °C for 72 h 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, were assessed using the North Carolina Differential Scale of 1 = no galls, 2 = 1–10 galls, 3 = 11–100 galls and 4 = >100 galls (Taylor and Sasser, 1978). *Rhizobium* nodules per root system were counted and weighed fresh.

Nematodes were extracted from total roots per plant by maceration and blending for 30 s in 1% NaOCl (Hussey and Barker, 1973) and passed through top-down nested 150-μm and 38-μm-pore sieves. Contents of the 25-μm mesh sieve were poured into 100-ml plastic containers for counting under a stereomicroscope. Soil per pot was thoroughly mixed and a 1 L soil sample was collected. Nematodes were extracted from a 250 ml soil subsample/pot using the modified sugar-floatation and centrifugation method (Jenkins, 1964). Nematode numbers from roots were converted to nematodes per total root system per plant, whereas soil nematode numbers were converted to 10 000 ml soil sample per pot.

2.5. Data analysis

Prior to analysis of variance (ANOVA), nematode data and nodulation numbers were transformed through $\log_{10}(x + 1)$ to homogenise the variances (Gomez and Gomez, 1984), but untransformed data were recorded. Data were subjected to ANOVA through the 2008 SAS software (SAS Institute, Inc., Cary, NC, USA.). Interactions between the 2009/2010 and 2010/2011 growing seasons for the variables measured were not significant ($P \leq 0.05$). Thus, data for the variables were pooled ($n = 20$) and subjected to statistical analysis. Data were partitioned using the sum of squares and the total treatment variation of sources of variation determined for each variable (Steyn et al., 2003). Unless otherwise stated, only treatments that were significant at the probability level of 5% were discussed.

3. Results

3.1. Effect on nematode numbers

Nematode × *Cucumis* interaction effects significantly (Table 1) contributed 2.2% and 2.3% to the total treatment variation (TTV) of J2s in roots and in soil, respectively, with nematode contributing 83.5% and 71.1% to TTV of the two variables (Table 2). In contrast, crude extracts of *C. myriocarpus* fruit contributed 12.7% and 26% to TTV of the two variables. Relative to nematode alone, *Cucumis* + nematode treatment reduced J2s in root and soil by 78% and 81%, respectively, with similar contributions to TTV of total juveniles and reproductive factors.

3.2. Effect on biomass

Nematode × *Bradyrhizobium* interaction significantly (Table 1) contributed 4% and 7% to TTV of fresh and dry shoot mass of