

# Mean Dosage Stimulation Range of Allelochemicals from Crude Extracts of *Cucumis africanus* Fruit for Improving Growth of Tomato Plant and Suppressing *Meloidogyne incognita* Numbers

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## Abstract

Successful utilisation of allelochemicals in management of plant-parasitic nematodes depends on their degree of phytotoxicity. Conventional methods of determining phytotoxicity are tedious, with inconsistent results. Plants respond to increased dosages of allelochemicals in a density-dependent growth pattern, which allows the use of the Curve-fitting Allelochemical Response Data computer-based model to determine the mean dosage stimulation range of used allelochemicals. The CARD modelling was used to determine the stimulation range of fermented dried crude extracts of wild cucumber (*Cucumis africanus*) fruit for improving growth of tomato (*Solanum lycopersicon*) plants, each infested with 1500 eggs and juveniles of the southern root-knot (*Meloidogyne incognita*) nematode. Dilutions at 0, 2, 4, 8, 16, 32 and 64% were applied weekly through irrigation system. At 56 days after treatment, CARD demonstrated density-dependent growth patterns as dosages increased. The mean dosage stimulation range of diluted fermented crude extracts, computed from CARD biological indices, was 2.64% dilution for tomato plant. Since at 2% dilution, the material reduced final nematode population density of *M. incognita* by 90%. The 2.64% was suitable for stimulation of tomato plant and suppression of nematode numbers.

**Keywords:** allelochemicals, *Cucumis africanus*, ground leaching technology, mean dosage stimulation range, *Meloidogyne incognita*

## 1. Introduction

Increased withdrawal of synthetic chemical nematicides from agrochemical markets exacerbated effects of the root-knot nematodes (*Meloidogyne* species) in tomato (*Solanum lycopersicon*) production (Mashela et al., 2011). Worldwide, *Meloidogyne* species continue to be the most devastating soil-borne pathogen in crop production. Crop losses prior to withdrawal of methyl bromide in 2005 were estimated at US\$125 billion (Chitwood, 2003). In Limpopo Province, South Africa, alternatives to methyl bromide in managing *Meloidogyne* species focused on uses of allelochemicals from crude extracts of selected plants using the ground leaching technology (GLT) system (Mashela et al., 2011). In this technology, crude extracts are applied in small quantities (0.2-0.7 t/ha) into soil around the stem during transplanting and suppressed *M. incognita* from 68-97% (12). The technology mitigated major drawbacks of conventional organic amendments in management of plant-parasitic nematodes (Stirling, 1991), such as (a) the use of excessively large quantities (10-250 t/ha), thus, reducing transport costs, (b) a waiting period to allow for microbial degradation, and therefore, avoiding negative period, (c) reduction of soil pH and, therefore, avoiding the unavailability of certain nutrient elements, and (d) inconsistent results on nematode suppression.

Widely used allelochemicals in GLT system include cucurbitacin A [cucumin (C<sub>27</sub>H<sub>40</sub>O<sub>9</sub>); leptodermin (C<sub>27</sub>H<sub>38</sub>O<sub>8</sub>)] from wild cucumber (*Cucumis myriocarpus* Naud.) (Jeffrey, 1978; Rimington, 1938) fruit and cucurbitacin B (C<sub>32</sub>H<sub>48</sub>O<sub>8</sub>) from wild watermelon (*Cucumis africanus* L. f.) fruit, both being indigenous to South Africa (Mashela et al., 2011). Cucurbitacins A and B are soluble and insoluble in water, respectively (Chen et al., 2005). Two major limiting factors in developing the two cucurbitacins for use in management of plant-parasitic nematodes were that (a) GLT system is labour intensive and, therefore, not cost-effective in large commercial farming systems, (b) the

two cucurbitacins are highly phytotoxic and (c) conventional methods for determining phytotoxicity in plants are tedious, with inconsistent results (Mashela et al., 2011).

Crude extracts of *C. myriocarpus* fruit at low dosages suppressed nematode numbers and improved growth of tomato plants (Mashela et al., 2011). Mafeo (2012) observed the existence of density-dependent growth patterns in plant variables as *C. myriocarpus* fruit dosages increased. Liu et al. (2003) developed the Curve-fitting Allelochemical Response Data (CARD) computer-based model, which quantified density-dependent growth patterns in biological organisms using seven biological indices, among which are those that assist in quantifying the stimulation, saturation and inhibition ranges (Salisbury & Ross, 1992).

A baseline study was previously conducted using cucurbitacin B through irrigation system in order to ameliorate the cost-ineffectiveness of this material in commercial farming system when used in GLT system (Pelinganga et al., 2012). Cucurbitacin B was extracted by fermenting fresh fruit of *C. africanus*, with a series of dilutions used through irrigation water. At harvest, cucurbitacin B had MDSR of 9% dilution, which also suppressed *M. incognita* race 2 numbers. However, fresh fruit of *C. africanus* have high incidence of post-harvest decay (Mphahlele et al., 2012), with unavailability challenges in areas where tomatoes are produced all-year-round. Successful use of crude extracts of dried *C. africanus* fruit in fermented form would mitigate against the unavailability issues. The objective of this study was to use CARD modelling to determine MDSR using dilutions of fermented crude extracts from dried *C. africanus* fruit for improving growth of tomato plants and suppressing *M. incognita* numbers.

## 2. Materials and Methods

### 2.1 Location and Preparation of Materials

The experiment was conducted at the greenhouse of the Plant Protection Skills Centre, University of Limpopo, South Africa (23°53'10"S, 29°44'15"E) in spring (August-October) 2011. Ambient day/night temperatures averaged 28/21°C, with maximum temperatures controlled using thermostatically-activated fans. Fruit of *Cucumis africanus* were harvested from field-grown plants, washed, cut into pieces and dried in air-forced ovens at 52°C for 72 h (Mashela et al., 2011) and ground in a Wiley mill to pass through a 1-mm-opening sieve. Approximately 40 g of crude extracts per 16 L tapwater in 20 L containers were fermented for 14 days at room temperature for pH to decline from 6.8 to 3.7 (Pelinganga et al., 2012) for preparation of treatment dilutions. When required, nematode inocula were prepared by extracting eggs and second stage juveniles (J2s) of *M. incognita* race 2 from roots of greenhouse-grown nematode-susceptible kenaf (*Hibiscus cannabinus* L.) in 1% NaOCl (Hussey & Barker, 1973). Twenty-cm-diameter plastic pots, at 0.3 m inter-row spacing and 0.25 m intra-row spacing, were each filled with 1 800 ml steam-pasteurised sand and Hygromix (Hygrotech, Pretoria North, South Africa) at 3:1 (v/v). Uniform four-week-old tomato 'Floradade' seedlings were transplanted and inoculated with 1 500 eggs and J2s of *M. incognita* race 2.

### 2.2 Experimental Design and Cultural Practices

Seven treatments, namely, 0, 2, 4, 8, 16, 32 and 64% dilutions were arranged in a randomised complete block design, with 10 replicates. Three days after transplanting, each plant was fertilised with 3 g 2:3:2 (22) to provide mg/ml water of 186 N, 126 K and 156 P, with 2 g 2:1:2 (43)-providing 0.35 N, 0.32 K and 0.32 P, 0.9 Mg, 0.75 Fe, 0.075 Cu, 0.35 Zn, 1.0 B, 3.0 Mn and 0.07 Mo. Four sets of Hadeco Moisture Meter (Hadeco, New Delhi, India) were inserted to 10-cm depths in randomly selected pots to monitor soil moisture tension. Plants were irrigated to full capacity using chlorine-free tapwater as soon as 50% moisture meter readings were below 2 units. Scouting for the greenhouse whitefly (*Trialeurodes vaporariorum* West.) was done weekly and plants sprayed with 1.33 ml Leybacid (a.i. fenthion 50% ml)/L water when population densities increased above 10 whiteflies per five randomly selected plants.

### 2.3 Data Collection

Flowers were counted weekly with pedicels marked to avoid recounting. At harvest, 56 days after inoculation, fruit of all sizes were recorded and plant height measured from soil surface to tip of flag leaf. Stems were severed at soil surface and stem diameter measured at 5 cm above severed ends using a digital vernier caliper. Shoots were oven-dried at 70°C for 72 h for dry shoot mass. Root systems were removed from pots, immersed in water to remove soil particles, blotted dry and weighed to facilitate the calculation of nematode density/total roots/plant. Roots were assessed for galls using the North Carolina differential scale where 0 = no galls, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls and 5 = > 100 galls/root system (Taylor & Sasser, 1978). Nematodes were extracted from total root system/plant by maceration and blending for 30 s in 1% NaOCl (Hussey & Barker, 1973). The material was passed through nested 61- and 38-µm opening sieves. Contents of the 38-µm-opening-sieve were

collected for separation of nematodes from fine debris using the sugar-floatation and centrifugation method (Jenkins, 1964). Soil in each pot was mixed and a 250 ml soil sample collected for nematode extraction using the sugar centrifugation and floatation method (Jenkins, 1964). Eggs and juveniles from root and soil samples were each counted using a stereomicroscope and converted to total root system per plant and total soil per pot, respectively. Root and soil nematodes from samples were converted to final nematode population density (Pf) in root, soil and then the total.

#### 2.4 Data Analysis

Data were subjected to analysis of variance (ANOVA) through the 2008 SAS software (SAS Institute, Inc., Cary, NC., USA). Flower, nematode and root gall data were transformed through  $\log_{10}(x + 1)$  to homogenise the variances (Gomez & Gomez, 1984). Sum of squares were partitioned to determine the contribution of sources of variation to the total treatment variation (TTV) in plant and nematode variables (Gomez & Gomez, 1984). Treatment mean separation was achieved using Waller-Duncan multiple range test at the probability level of 5% and further subjected to CARD model to generate appropriate biological indices (Liu et al., 2003). Then, after adjusting  $R_h$  for  $D_m$  in plant variables, MDSR was computed by halving the sum of  $D_m$  and adjusted  $R_h$ .

Unless otherwise stated, only treatments significant at the probability level of 5% were discussed.

### 3. Results

#### 3.1 Effect on Plant Growth

Treatment effects were significant for dry shoot mass, dry root mass, plant height, stem diameter and nematode numbers. In dry shoot mass, dry root mass, plant height, stem diameter, nematode in roots, nematode in soil and total nematodes the dosage levels contributed 42%, 38%, 28%, 19%, 84%, 44% and 82% to the total treatment variation (TTV), respectively (Table 1). Dosage levels had no effect on numbers of flowers and fruit mass (data not shown).

Plant growth had density-dependent growth patterns as dosage levels increased (Figure 1). Biological indices in CARD model were strongly explained by dosage levels as shown by coefficients of variation ( $R^2$ ) of dry shoot mass, dry root mass, plant height and stem diameter at 96%, 97%, 99% and 90%, respectively (Table 2). Various plant organs had different biological indices except for k values, which were for each organ equivalent to unity. Overall, MDSR dilution of fermented crude extracts of *C. africanus* fruit for tomato was at 2.64%.

#### 3.2 Effect on Nematode Suppression

Relative to untreated control, dosages of crude extracts from *C. africanus* fruit reduced nematode numbers from 85-97%, 45-96% and 78-97% for root, soil and total nematodes, respectively (Table 3). The impact of dilutions appeared to be inversely proportional to the dosage level in all measurement units. In untreated control, galling was higher than in treated plots, while the latter were not different from one another.

### 4. Discussion

#### 4.1 Plant Growth

High coefficients of determination ( $R^2$ ) for CARD models in four plant variables suggested strong density-dependent relationships between growth of tomato and increasing dilutions of crude extracts of *C. africanus* fruit. Generally, k values of unity in all organs suggested that in tomato plant, the assessed organs had similar sensitivities to dilutions of allelochemicals from fermented crude extracts of *C. africanus* fruit. Plant sensitivity is indirectly proportional to k values, with zero suggesting the highest sensitivity to allelochemicals used, while high k values suggested decreased sensitivities (Liu et al., 2003). Observed k values on tomato in this study were different from zero values observed for dry shoot mass, plant height and stem diameter under dilutions from fresh fermented *C. africanus* fruit (Pelinganga et al., 2012). In GLT, Mafeo (2012) showed that k values of tomato seedlings ranged from 9 to 20 depending on the investigated organ. Apparently, k values are affected by various factors, which may include fermentation of dried versus fresh materials, fermented versus unfermented, age of the test plant and/or organ of the test plant.

Overall,  $\sum k$  value for whole tomato plant in the current study was 4, while that for fresh fermented fruit of *C. africanus* was unity (Pelinganga et al., 2012), which is another cue suggesting that fermented fresh fruit of *C. africanus* are more phytotoxic to tomato plants than fermented dried fruit. Grinding concentrates potent chemicals which are responsible for stimulation of plant growth at low levels. However, our results cannot be compared with those of Mafeo (2012) since plants were exposed to unfermented crude extracts for 18 days, while in this and other studies (Pelinganga et al., 2012) exposure time was for 56 days. Thus, proper trials using dry and equivalent fresh

materials are required to make reliable inferences about the sensitivity (k) of crops to fermented crude extracts of *Cucumis* fruits.

Stimulation of plant growth at low dosages of crude extracts of *Cucumis* fruits appears to be universal, as shown in various organisms using various types of allelochemicals (Liu et al., 2003). The phenomenon was previously observed in eight and ten monocotyledonous and dicotyledonous plants, respectively (Mafeo, 2012). Certain cucurbitacins from *Cucumis* fruits were shown to have anticancer activities at high dosages, which however, had nonspecific cytotoxicity, while at low dosages the materials stimulated cell division (Chen et al., 2005; Geissman, 1964). MDSR of 2.64% from fermented crude extracts in this study is much lower than that derived from fermented fresh fruit of *C. africanus*, which was approximately 9% (Pelinganga et al., 2012). The disparities in MDSR from dried and fresh materials could explain why fermented fresh fruit of *C. africanus* in Pelinganga et al. (2012) had much lower k values than those of fermented dried fruit in the current study. The recommended MDSR is much lower than  $D_0$  and  $D_{50}$  inhibition biological indices, which, in this study were 9.76% and 1181.71%, respectively. Thus, the MDSR also ensures that a phytotoxic dosage level is not incorrectly recommended.

#### 4.2 Nematode Suppression

Suppression of nematodes in all test dosages confirmed the nematicidal properties of allelochemicals in *C. africanus* (Mashela et al., 2011). Observed nematode suppression in both root and soil were quite high. The decline in the efficacy of the dilutions in nematode suppression could also be attributed to the phytotoxicity of the materials, which inadvertently affect infection sites and therefore, nematode numbers. Nematode suppression using fermented crude extracts in our study was comparable to that observed when using fermented fresh fruit of *C. africanus* (Pelinganga et al., 2012). Apparently, drying at 52°C for 72 h had no effect on cucurbitacin B, which had been identified as a potent allelochemical that confers nematicidal properties in crude extracts from *C. africanus* fruit.

### 5. Conclusion

In dried form, MDSR from fermented crude extracts of *C. africanus* fruit was established for tomato plants and suppression of *M. incognita* race 2 population densities. The derived MDSR should, accordingly be validated under various environmental conditions using different tomato cultivars. Successful validation would enable the cultivation of *C. africanus* in summer and process the crude extracts from fruit and then store the materials in dried form for use during off-seasons.

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