African Journal of Biotechnology Vol. 11(52), pp. 11407-11413, 28 June, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.138
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Baseline information on using fermented crude extracts from *Cucumis africanus* fruit for suppression of *Meloidogyne incognita* and improving growth of tomato plants

O. M. Pelinganga, P. W. Mashela*, B. Nzanza and M.S. Mphosi

Department of Soil Science, Plant Production and Agricultural Engineering, School of Agricultural and Environmental Sciences, University of Limpopo, Private Bag X1106, Sovenga 0727, South Africa.

Accepted 13 June, 2012

Bio-pesticides, when used as a post-planting pesticide, are limited by their potential ability to suppress the pest and their degree of phytotoxicity. Baseline information on the suitability of fermented crude extracts (FCE) of *Cucumis africanus* fruit as a post-planting bio-nematicide was determined on *Meloidogyne incognita* and growth of tomato in pot trials. Seedlings were inoculated with 1,130 eggs and juveniles/pot, while FCE were applied weekly at 0, 10, 20, 30, 40, 50 and 60% dilutions. At harvest, nematode and plant data were subjected to analysis of variance and the curve-fitting allelochemical response data (CARD) computer-based model, respectively. Relative to inoculation level, final nematode population density (Pf) at the same level of inoculation was reduced, while increasing dosages had no effect on Pf and reproductive factor values. Since increasing dosages had no effect on Pf, the material could possibly still reduce this variable when applied below 10% dilutions. Dosages used were phytotoxic to tomato plants, but CARD model demonstrated that the material stimulated plant growth at dosages below 10%. In conclusion, baseline information from CARD model suggested that FCE from *C. africanus* fruit have the potential to serve as a bio-nematicide and bio-fertiliser on tomato provided the material was applied below 10% dilutions.

Key words: *Cucumis africanus*, bionemagation, effective microbe organisms, ground leaching technology, root-knot nematode.

INTRODUCTION

International agreements to withdraw the highly effective methyl bromide (MB) and related fumigant nematicides due to their ozone-depleting properties have had deleterious consequences in management of plant-parasitic nematodes (Mashela et al., 2011). Global crop yield losses due to nematodes prior to the 2005 MB cut- off withdrawal date had been estimated at US$125 billion/ annum (Chitwood, 2003), with percentage estimates ranging from 6 to 20% (Ferraz and Brown, 2002). In certain crops like watermelon (*Citrullus lanatus*), which have no resistant genotypes to the root-knot nematodes (*Meloidogyne* species), yield losses ranged from as high as 50% to total crop failure (Lamberti, 1979). Current estimated annual crop losses due to nematode damage in South Africa stand at 14% (Swart, 2010). Detailed estimates of crop losses due to nematodes suggested that this pest was a major contributor towards overall yield losses in the USA (Koenning et al., 1999).

Use of organic amendments as alternative to MB received much attention after the adoption of the Montreal

*Corresponding author. E-mail: phatu.mashela@ul.ac.za.

Abbreviations: MB, Methyl bromide; **GLT,** ground leaching technology; **FCE,** fermented crude extracts; **EM,** effective microbe organisms; **CARD,** curve-fitting allelochemical response data.

protocol (Bello, 1998). Unfortunately, large quantities (10 to 250 t/ha) of organic amendments were required to effectively suppress nematode numbers and therefore, the technology was uneconomic in terms of availability and transport of the materials (Mankau, 1968; Mankau and Minter, 1962; McSorley and Gallaher, 1995; Muller and Gooch, 1982; Rodriguez-Kabana, 1986; Stirling, 1991). In addition to inconsistent results in nematode supperssion, most organic amendments reduced soil pH (Mashela et al., 2010; Muller and Gooch, 1982; Stirling, 1991), resulting in unintended consequences of unavailability and/or supra-availability of certain elements in the soil (Bohn et al., 1985). The induced imbalances invariably manifested as deficiencies and/or toxicities of nutrient elements in crops.

Attempts to mitigate drawbacks of conventional organic amendments in nematode suppression led to the widespread research and development of the ground leaching technology (GLT) system in marginal communities of Limpopo Province, South Africa (Mashela, 2002; Mashela and Mphosi, 2002; Mashela and Nthangeni, 2002; Mashela et al., 2011). Unfermented small quantities (0.20 to 0.71 t/ha) of crude extracts of wild cucumber (*Cucumis myriocarpus*) fruit were successfully used to suppress the southern root-knot nematode (*Meloidogyne incognita*), with evidence of fertiliser effect on tomato (*Solanum lycorpersicum*) plants (Mashela, 2002; Mashela and Mphosi, 2002; Mashela et al., 2011; Mphosi et al., 2004). In addition to consistent results, the material did not reduce soil pH at the dosages used. However, the GLT system, as currently used by small-scale farmers is labour-intensive and would therefore, not be cost-effective in large-scale commercial farming systems. Initial attempts to use powdered materials from dried *C. myriocarpus* fruit were not successful due to phytotoxicity, repugnant smell and blockages of dripline holes (Land Bank Chair of Agriculture – University of Limpopo, unpublic. data). The smell and blockage challenges were eventually resolved using effective microbe organisms (EM) and the sieving of fermented materials, respectively.

After Mafeo (2011) had successfully used the Liu et al. (2003) computer-based model to derive non-phytotoxic unfermented dosages of crude extracts of *C. myriocarpus* fruit as pre-emergent bio-nematicide, the idea of applying the material through irrigation system, referred to as boti-nemagation, was revisited (Mashela et al., 2011).

Generally, biological systems respond to extrinsic or intrinsic factors in accordance to density dependent growth patterns, which are characterised by stimulation, saturation and inhibition phases (Salisbury and Ross, 1992). Conventional methods of determining density dependent growth patterns are tedious, with inconsistent results (Inderjit, 2001). The curve-fitting allelochemical response data (CARD) computer-based model was developed to quantify density dependent growth patterns in biological systems (Liu et al., 2003). In the CARD models, density dependent growth patterns are characterised by seven biological indices, namely: (1) threshold stimulation (D_m) - the dosage at which the allelochemical begins to have a measurable stimulating effect on plant growth, (2) saturation point (R_h) - the dosage at which growth remains constant prior to decreasing, (3) 0% inhibition (D_0) - the end-point dosage of R_h where the allelochemical has a zero effect on growth reduction, (4) 50% inhibition (D_{50}) - the dosage where the allelochemical inhibits growth by 50%, (5) 100% inhibition(D_{100}) - the dosage where the allelechemical inhibits growth by 100%, (6) k - the number of In(D + 1) transformations that serve as a biological indicator of the degree of sensitivity with relation to stimulation or inhibition to allelochemicals and (7) R^2 - the coefficient of determination (Liu et al., 2003).

The distance between D_m and R_h had been referred to as the stimulation range (Mafeo et al., 2011a, b), which for the first time explained the previously observed fertiliser effect on crops when using crude extracts of *C. myriocarpus* fruit (Mashela, 2002). Generally, the limiting factor in developing any pesticide intended for post-plant use is its degree of phytotoxicity. The k value in the CARD model is important in establishing whether a material is phytotoxic or non-phytotoxic (Mafeo et al., 2011b). Usually, k values start from zero and increased as discrete numbers when the sensitivity of the plant to the test material decreased (Liu et al., 2003). In other words, the sensitivity of the test plant to the bio-nematicide is inversely proportional to the k values. Thus, this biological index could serve as an indicator of whether selected dosages of a particular botanical were suitable or not for use as a post-planting bio-nematicide. The objective of this study was to determine baseline information for using EM-fermented crude extracts (FCE) of *C. africanus* fruit in suppression of *Meloidogyne* species and improving growth of tomato plants.

MATERIALS AND METHODS

Location of study and preparation

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 autumn (August to October) 2011. Ambient day/night temperatures averaged 28/21°C, with maximum temperatures controlled using thermostatically-activated fans. Other greenhouse variables such as relative humidity, photosynthetically active radiation and solar radiation were not measured. *Cucumis africanus* fruit were collected from locally cultivated plants after fruit maturity (Mafeo and Mashela, 2009) and cut into pieces. Approximately, 500 g fresh materials were fermented in 20 L-sealed plastic containers with 16 L chlorine-free tapwater. Allowance for released CO_2 to escape from the container was provided through an airtight 5 mm-diameter tube with one end glued to a hole on the lid of the 20 L container, while the outlet end dangled into a litre bottle half-filled with tapwater.

Approximately, 300 ml molasses, 100 g brown sugar and 300 ml EM was added into each container. After a 14 day incubation period, when pH was at 3.7 or less, FCE were passed through a 2

Table 1. Influence of diluted fermented crude extracts of *Cucumis africanus* fruit on final nematode population density (Pf), percentage impact and reproductive factor (RF) of *Meloidogyne incognita* race 2 (n = 30).

Dilution (%)	(Pi)	Pf/total root	Pf/total soil	Pf	Impact (%)[y]	(RF)[z]
10	1130	28	65	93	92**	0.082
20	1130	69	151	220	80**	0.195
30	1130	69	65	134	88**	0.118
40	1130	66	43	109	90**	0.096
50	1130	37	130	167	85**	0.147
60	1130	58	65	123	89**	0.108

[y]Impact (%) = [(Treatment/Pi) ─ 1) × 100], where ** implied that Pi and Pf were significantly different at 5% level of probability according to student t-test. [z]Reproductive factor = Pf/Pi.

mm-opening mesh sieve into a 20 L container for subsequent preparations of the required dilutions on irrigation days. Consequently, for each weekly application, new dilutions were prepared from freshly fermented crude extracts. When required, nematode inoculums were prepared by extracting eggs and juveniles of *M. incognita* race 2 from roots of greenhouse-grown nematode-susceptible tomato cv. 'Floradade' in 1% NaOCl (Hussey and Barker, 1973).

Experimental design and cultural practices

Briefly, 20 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 seedlings cv. 'Floradade' were transplanted and inoculated with 1, 300 eggs and juveniles of *M. incognita* race 2. Seven treatments, viz. 0, 10, 20, 30, 40, 50 and 60% dilutions were arranged in a randomised complete block design, with 5 replicates. Three days after transplanting, each plant was fertilised with 3 g 2:3:2 (22) to provide a total of 186 mg N, 126 mg K and 156 mg P per ml water and 2 g 2:1:2 (43) – providing 0.35 mg N, 0.32 mg K and 0.32 mg P, 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 per ml water. 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% of the moisture meters had readings below 2 units. Plants were scouted for the greenhouse whitefly (*Trialeurodes vaporariorum)* and sprayed with 1.33 ml Leybacid (active ingredient fenthion 50% ml)/L water when population densities increased above 10 whiteflies per five randomly selected plants.

Data collection

Flowers were occasionally counted with pedicels marked to avoid recounting. At harvest, 56 days after inoculation, fruit/plant was collected and plant height measured from the soil surface to the tip of the flag leaf. Stems were cut off at the soil surface and the stem diameters measured at 5 cm above the severed ends using a digital vernier caliper. Fresh fruit were weighed, 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/total roots/plant. Root galling was based on the scale of 0 to 5, in which 0 = no galls, 1 = 1 to 2 galls, 2 = 3 to 10 galls, 3 = 11 to 30 galls, 4 = 31 to 100 galls and 5 = >100 galls/root system (Taylor and Sasser, 1978). Nematodes were extracted from total root system/plant by maceration and blending for 30 sec in 1% NaOCl (Hussey and Barker, 1973). The material was passed through nested 61- and 38 µm mesh sieves. The contents of the 38-µm mesh sieve were collected for further separation of nematodes from debris using the sugar-floatation and centrifugation method (Jenkins, 1964). Soil in each pot was thoroughly mixed and a 250 ml soil sample collected for nematode extraction using the sugar centrifugation and flotation 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. Final nematode population density (Pf) allowed for calculation of reproductive factor (RF = Pf/Pi) values, where Pi was initial nematode population density.

Data analysis

Data were subjected to analysis of variance (ANOVA) through the 2008 SAS software (SAS Institute, Inc., Cary, NC., U.S.A.). Flower, nematode and root gall data were transformed through $\log_{10}(x + 1)$ to homogenise the variances (Gomez and Gomez, 1984), but untransformed means were reported. Treatment mean separation was achieved using Waller-Duncan multiple range test at the probability level of 5%. Mean plant variables were subjected to the CARD model generating the regression curve estimations using quadratic equation: $Y = b_2x + b_1x + a$, where Y = plant variable value and x computed from $x = -b_1/2b_2$, where x = the optimum dosage level, which is a dilution value where saturation sets in (Salisbury and Ross, 1992), along with the biological indices, which includes D_m, R_h, D_0, D_{50}, D_{100}, k and R^2 (Liu et al., 2003). Unless otherwise stated, only treatments that were significant at the probability level of 5% were discussed.

RESULTS AND DISCUSSION

Relative to Pi, Pf at the same dosages was reduced, whereas increasing dosages from 10 to 60% had no effect on Pf (Table 1). The impact of dosages on Pf at the same level ranged from 80 to 92%, while RF values at all levels were less than unity, but did not differ from 10 to 60% dosages. In 0% dilution, root galls were well-developed, whereas in 10 to 60% dilutions they were poorly-developed. However, the variation in root galls as dosages increased was similar to Pf (data not shown).

Table 2. Biological indices of flower number, fresh fruit mass, dry shoot mass, fresh root mass, plant height and stem diameter of tomato exposed to diluted fermented crude extracts of *Cucumis africanus* fruit at 56 days after the treatments (n = 35).

Biological index	Flower number	Fruit yield mass	Dry shoot mass	Fresh root mass	Plant height	Stem diameter
Threshold stimulation (D_m)	3.435	21.845	1.012	10.724	1.470	18.639
Saturation point (R_m)	2.123	0.323	0.002	0.758	0.025	0.459
0% inhibition (D_0)	18.672	43.690	0.005	21.449	2.939	37.278
50% inhibition (D_{50})	65.697	52.941	51.414	50.291	65.204	69.351
100% inhibition (D_{100})	150.700	60.000	73.100	65.600	91.600	87.900
R^2	0.97	0.90	0.92	0.92	0.88	0.97
K	1	0	0	0	0	0

Sensitivity ranking: $\sum k = 1$.

Threshold stimulation (D_m) for various organs differed from 1 unit in dry shoot mass to 22 units in fruit yield mass, with saturation points (R_h) being attained within almost a fraction of the respective D_m values (Table 2). Using the flower number to describe the observed D_m values, D_0 occurred at 3.435 units and from D_0 to R_m additional 2.123 units were required for a total dosage of 5.558 (3.435 + 2.123) units from the untreated control. Similarly, from untreated control to D_{100} a total dosage of 154.135 (3.435 + 150.700) units were required.

Graphic presentation examples of fruit yield and dry shoot mass against FCE dosages demonstrated that at low dosages, the material stimulated growth, while at high dosages, inhibition occurred (Figure 1). Transformation levels for flower number decreased from k = 1 (R^2 = 0.97), with the model ceasing to run at k = 2 (R^2 = 0.81). In fruit yield mass, dry shoot mass, fresh root mass, plant height and stem diameter, the best fits to the CARD lines were achieved at k = 0 for each variable (Table 2). Sensitivity ranking (Σk) of tomato exposed to the test material was equivalent to unity. The R^2 values for all the models ranged from 87 to 97%. Computed optimum response dosage (CORD) values (Table 3), derived from the quadratic relationships for plant variables were more or less equivalent to those derived from the CARD model.

Increasing FCE dosages of *C. africanus* fruit suppressed population densities of *M. incognita* race 2 on tomato plants equally at all levels. This suggested that fermenting the material did not interfere with the LD_{50} of the potent chemical that conferred nematicidal properties, which had been established as 1.6 mg kg^{-1} (Mashela et al., 2011). Also, the LD_{50} to nematodes in this test material, as in other pesticides, cannot be increased by increasing the dosage. The potent chemical in *C. africanus* fruit had been identified as cucurbitacin B ($C_{32}H_{48}O_8$), which is insoluble in water (Chen et al., 2005; Jeffrey, 1978). Cucurbitacin B is equally distributed in all parts of *C. africanus* plant (Jeffrey, 1978) and was previously shown to have minimum inhibition concentration of 7 µg/ml for *M. incognita* and the citrus nematode, *Tylenchulus semipenetrans* (Mashela et al., 2011). In this study, the fact that different FCE dosages on nematode suppression were not different suggested that the phytotoxicity of the material on tomato was the limiting factor in the utility of this material as a potential bio-nematicide. Incidentally, dosages that are suppressive to nematode numbers, but phytotoxic to the protected plant, would not be useful, particularly when applied as a post-planting bio-nematicide.

Generally, at low dosages, FCE of *C. africanus* fruit stimulated growth of tomato plants, while at high dosages the material inhibited plant growth, which agreed with the primary tenets of density dependent growth patterns in biological systems (Liu et al., 2003). The CARD model in our study confirmed responses of chive (*Allium schoenoprasum*), leek (*Allium ampeloprasum*) and onion (*Allium cepa*) when exposed to unfermented crude extracts of *C. myriocarpus* fruit (Mafeo et al., 2011a). Similar responses were observed in the family *Gramineae* (Mafeo et al., 2011b). Crude extracts of *C. myriocarpus* fruit were suitable for use as pre-emergent bio-nematicide when the dosage was equivalent to the mean of the stimulation range [(D_m + R_h)/2], referred to as the 'mean dosage stimulation range' (MDSR). When used at MDSR values, Mafeo (2011) demonstrated that crude extracts of *C. myriocarpus* fruit suppressed Pf from 88 to 99%, with fertiliser effect on various crops.

In the CARD model, as k increased from zero, generally R^2 initially increased to a peak, where k = i and then decreased from i + 1 transformations until the model ceased running (Liu et al., 2003). In our study, as dosages increased from 10 to 60%, k remained at zero for all variables except for flower number where the model started and ceased running at k equalled 0 and 2, respectively, but with the highest R^2 being at k = 1. The k = 0 value agreed with that observed for the radicle length in maize seedlings exposed to crude extracts of *C. myriocarpus* fruit over 18 days (Mafeo et al., 2011b). In other words, with the exception for the flower number in

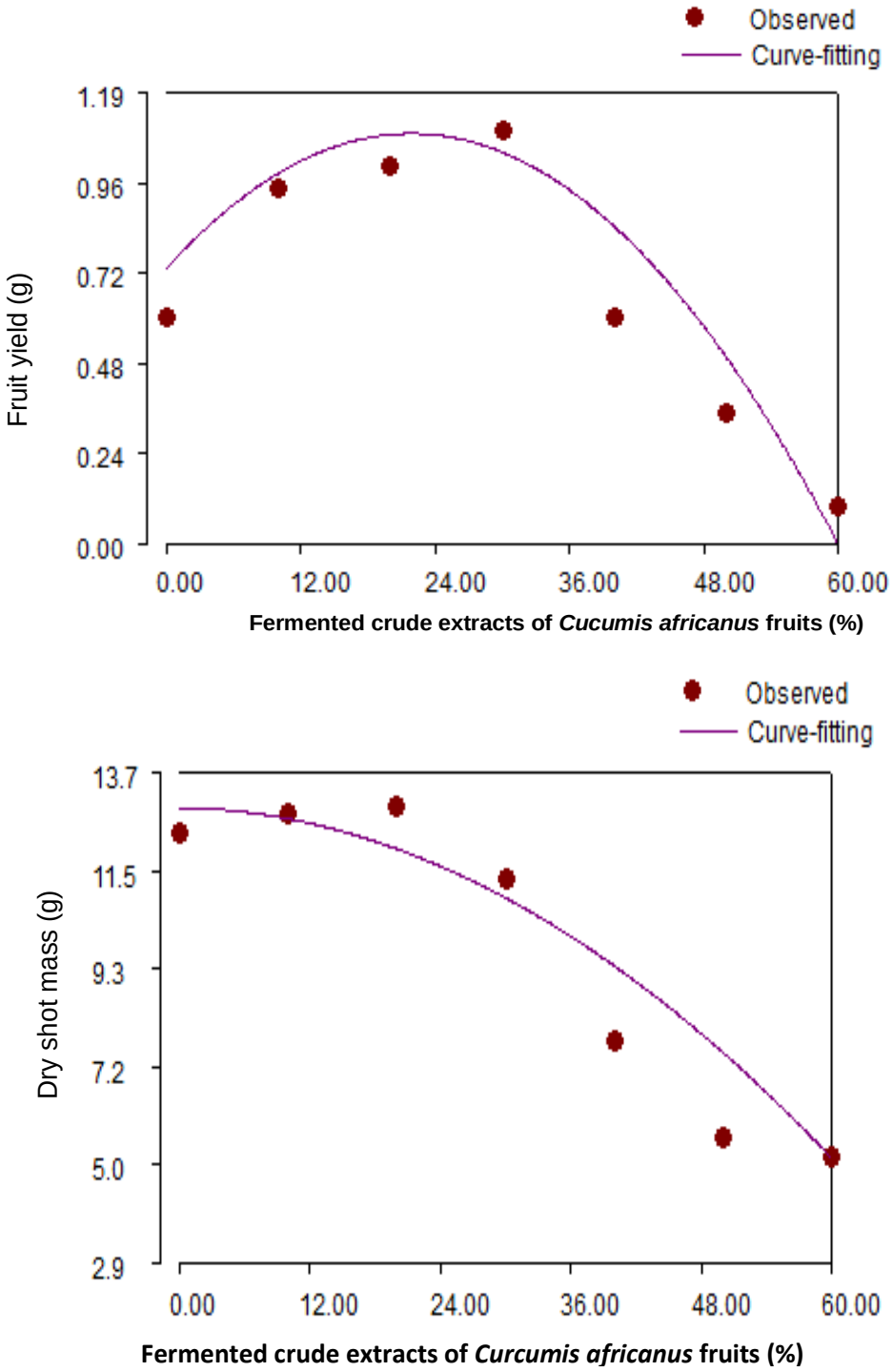

Figure 1. Responses of dry shoot mass, fresh root mass, plant height, stem diameter, flowers and fruit to dosages of fermented crude extracts of *Cucumis africanus* fruit at 56 days after initiating the treatments (n = 35).

our study, the sensitivities of tomato organs as dosages increased were quite high and organ non-specific. However, in tomato seedlings exposed to crude extracts of *C. myriocarpus* fruit over 18 days, k differed from organ to organ, with k for hypocotyl diameter, hypocotyl length, epicotyl length and seedling height being equal to

Table 3. Quadratic relationship, coefficient of determination and computed optimum response dosage for variables of tomato from the Curve-fitting Allelochemical Response Data against diluted fermented crude extracts of *Cucumis africanus* fruit at 56 days after treatments (n = 35).

Plant variable	Quadratic relationship	R^2	CORD $(x)^z$	P ≤
Flower number	$-0.957x^2 + 2.850x + 9.813$	0.97	1.489	0.01
Fruit yield mass (g)	$-0.003x^2 + 0.131x + 5.911$	0.90	21.833	0.01
Dry shoot mass (g)	$-0.002x^2 + 0.005x + 12.880$	0.92	0.357	0.01
Fresh root mass (g)	$-0.007x^2 + 0.141x + 19.115$	0.92	10.071	0.01
Plant height (cm)	$-0.011x^2 + 0.033x + 92.183$	0.88	1.500	0.01
Stem diameter (mm)	$-0.001x^2 + 0.049x + 5.877$	0.97	24.500	0.01

Calculated optimum response dosage (x) = $-b_1/2b_2$, where for flower number $b_1 = 2.850$ and $b_2 = -0.957$.

15, 7, 20 and 9 units, respectively (Mafeo, 2011). In that 18 days study (Mafeo, 2011), sensitivities of organs to the test material in emerging tomato seedlings were organ-specific, with the hypocotyl length being the most sensitive and the epicotyl length the least sensitive. The ∑k of unity in our study suggested that the tomato plant was highly sensitive to FCE of *C. africanus* fruit when compared to unfermented crude extracts of *C. myriocarpus* fruit, where ∑k in tomato was 51 (Mafeo, 2011). In addition to different Cucumis species having different potent chemicals, the exposure time and age of the test plants could also play a role, since in the other study (Mafeo, 2011) seedlings were exposed for 18 days when compared to our 56-day exposure time which started with four-week old seedlings. In the 18 days study (Mafeo, 2011), tomato seedlings were the least sensitive (∑k = 51) to crude extracts of *C. myriocarpus* fruit, while eggplant (*Solanum melongena*) seedlings were the most sensitive (∑k = 9).

On the whole, the results of this study suggested that increasing FCE dosages of *C. africanus* fruit from 10 to 60% was highly toxic to tomato plants as shown by the overall sensitivity ranking of unity. The CARD model suggested that the stimulation ranges of diluted FCE of *C. africanus* fruit for tomato plants were below the 10% dilution, with CORD values derived from the quadratic relationship using the $x = -b_1/2b_2$ relation agreeing with D_m instead of R_h values. This was further evidenced by the fact that the selected dosages were quite high. The CARD model demonstrated once again the importance of choosing appropriate dosages in phytotoxicity studies (Mafeo, 2011). For instance, when the dosages are already above the saturation point, the relation could be depicted by a negative linear relationship, whereas dosages prior to the saturation point conferred positive linear relationships (Mamphiswana et al., 2010). In plant-parasitic nematodes, Pofu et al. (2010) explained the observed quadratic relationship between RF and Pi on the basis of the equilibrium point, which in this study is analogous to R_h point. In the current study, most of the dosages were already on the R_h point. However, due to the robust iterative nature of the CARD model, it became evident that appropriate FCE dosages of *C. africanus* fruit would be below 10% dilution. Consequently, in order to avoid phytotoxicity, while the primary objective of suppressing nematode numbers is achieved, dosages in the range of 0, 2, 4, 6 and 8% dilutions should be tested for phytotoxicity and nematode suppression.

Conclusion

Diluted FCE of *C. africanus* fruit retained their nematicidal properties as shown by their capability to suppress numbers of *M. incognita* race 2. However, at the used dosages the material was highly phytotoxic to tomato plants. Consequently, in order to avoid phytotoxicity, while achieving nematode suppression, dosages in the range of 0, 2, 4, 6 and 8% could provide the optimum dosage for achieving nematode suppression and fertiliser effect on plant growth.

ACKNOWLEDGEMENTS

The authors are indebted to the Department of Science and Technology, the Land Bank Chair of Agriculture – University of Limpopo and the Flemish Interuniversity Council (VLIR) for providing financial support.

REFERENCES

Bello OA (1998). Biofumigation and integrated crop management. In: Bello OA, Gonzalez JA, Arias M and Rodriguez-Kabana R (eds), Alternatives to methyl bromide for the Southern European Countries DG XI, EU, CSIC, Madrid, Spain.

Bohn HL, McNeal BL, O'Connor GA (1985). Soil chemistry. Wiley, New York.

Chen JC, Chiu MH, Nie RL, Cordell GA, Qiu SX (2005). Cucurbutacins and cucurbitane glycosides: Structures and biological activities. Nat. Prod. Rep., 22: 386-399.

Chitwood DJ (2003). Research on plant-parasitic nematode biology conducted by the United States Department of Agricultural Research Services. Pest Manag. Sci., 59: 748-753.

Ferraz LCCB, Brown DJF (2000). An introduction to nematodes: Plant nematology. Pensoft, Sofia, Bulgaria.
Gomez KA, Gomez AA (1984). Statistical procedures for agricultural research. Wiley, New York.
Hussey RS, Barker KR (1973). A comparison of methods of collecting inoculants *Meloidogyne* species including a new technique. Plant Dis. Rep., 57: 1025-1028.
Inderjit (2001). Soils: Environmental effect on allelochemical activity. Agron. J., 93: 79-84.
Jeffrey C (1978). Cucurbitaceae. In: Launert E (ed.), Flora Zambesiac. Managing Committee, London, UK.
Jenkins WR (1964). A rapid centrifugal-floatation technique for separating nematodes from soil. Plant Dis. Rep., 48: 692.
Koenning SR, Overstreet C, Noling JW, Donald PA, Becker JO, Fortnum BA (1999). Survey of crop losses in response to phytoparasitic nematodes in the United States for 1994. J. Nematol., 31: 587-618.
Lamberti F (1979). Chemical and cultural control. In: Lamberti F, Taylor CE (eds), Root- knot nematodes (*Meloidogyne* species) systematics, biology and control. Academic press, London. pp. 403-423
Liu DL, An M, Johnson IR, Lovett JV (2003). Mathematical modeling of allellopathy. III. A model for curve-fitting allellochemical dose responses. Non-linearity Biol. Toxicol. Med., 1: 37-50.
Mafeo TP (2011). Responses of economically important crops to crude extracts of *Cucumis* fruit when used as pre-emergent bio-nematicide. PhD Thesis, University of Limpopo, Sovenga, South Africa.
Mafeo TP, Mashela PW, Mphosi MS (2011a). Sensitivity of selected Alliaceae seedlings to crude extracts of *Cucumis myriocarpus* fruits. Afr. J. Agric. Res., 6: 158-164.
Mafeo TP, Mashela PW, Mphosi MS, Pofu KM (2011b). Modelling responses of maize, millet and sorghum seedlings to crude extracts of *Cucumis myriocarpus* fruit as pre-emergent bio-nematicide. Afr. J. Agric. Res., 6: 3678-3684.
Mafeo TP, Mashela PW (2009). Responses of germination in tomato, watermelon, butternut, Squash to a *Cucumis* bio-nematicide. Am. Eur. J. Agric. Environ. Sci., 6: 215-219.
Mamphiswana ND, Mashela PW, Mdee LK (2010). Distribution of total phenolics and antioxidant activity in fruit, leaf, stem and root of *Monsonia burkeana*. Afr. J. Agric. Res., 5: 2570-2575.
Mankau R (1968). Reduction of root-knot disease with organic amendments under semi-field conditions. Plant Dis. Rep., 52: 315-319.
Mankau R, Minteer RM (1962). Reduction of the soil population of the citrus nematode by addition of organic matter. Plant Dis. Rep., 46: 375-376.
Mashela PW, De Waele D, Pofu KM (2011). Use of indigenous *Cucumis* technologies as alternative to synthetic nematicides in management of root-knot nematodes in low-input agricultural farming systems: A review. Sci. Res. Essays, 6: 6762-6768.
Mashela PW, Shimelis HA, De Waele D, Mokgalong MN, Mudau N, Ngobeni GL (2010). Fever tea (*Lippia javanica*) as a root-knot nematode suppressant in tomato production. Afr. Plant Prot., 16: 1-6.
Mashela PW (2002). Ground wild cucumber fruits suppress numbers of *Meloidogyne incognita* on tomato in microplots. Nematropica, 32: 13-19.
Mashela PW, Mphosi MS (2002). Wild cucumber fruit residues reduce population densities of *Meloidogyne incognita* on tomato plants. Afr. Plant Prot., 8: 84.
Mashela PW, Nthangeni ME (2002). Efficacy of *Ricinus communis* fruit meal with and without *Bacillus* species on suppression of *Meloidogyne incognita* and growth of tomato. J. Phytopathol., 150: 399-402.
McSorley R, Gallaher RN (1995). Effect of yard waste compost on plant-parasitic nematode densities in vegetable crops. Suppl. J. Nematol., 27: 545-549.
Muller R, Gooch PR (1982). Organic amendments in nematode control: An examination of the literature. Nematropica, 12: 319-326.
Mphosi MJ, Mashela PW, Shakwane MK (2004). Efficacy of *Cucumis myriocarpus* with and without *Bacillus* species on suppression of *Meloidogyne incognita*. Afr. Plant Prot., 10: 130.
Pofu KM, Mashela PW, Mphosi MS (2010). Responses of *Cucumis africanus* and *Cucumis myriocarpus* to *Meloidogyne incognita* race 2 under microplot conditions. Int. J. Nematol., 20: 113-118.
Rodriguez-Kabana R (1986). Organic and inorganic nitrogen amendments to soil as nematode suppressant. J. Nematol., 18: 129-135.
Salisbury FB, Ross CW (1992). Plant physiology. Wadsworth, Belmont, USA.
Stirling GR (1991). Biological control of plant-parasitic nematodes. CABI, Wallingford, UK.
Swart A (2010). Research on Nematodes. Agricultural Research Council, http://www.arc.agric. Accessed 28 October 2011.
Taylor AL, Sasser JN (1978). Biology, identification and control of root-knot nematodes *(Meloidogyne* species*)*. North Carolina State University Press, Raleigh, North Carolina.