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Article Original

Development of a bionematicide from crude extracts of cucumis africanus fruit

Desarrollo de bionematicida a partir de extractos crudos del fruto Cucumis africanus

Osvaldo Manuel Pelinganga, Doctor of Philosophy in Plant Protection, Assistant Professor, Instituto Superior Politécnico do Cuanza Sul. Sumbe, Angola, osvaldopelinganga_7@hotmail.com

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Abstract

A 14-day experiment was conducted at the University of Limpopo laboratory of nematology to determine the optimum quantity of plant material required for producing a bionematicide from *Cucumis africanus* fruit with a pH value below 3.5. Seven treatments comprising of 0, 20, 40, 80, 160, 320 and 640 g of ground fruit of *Cucumis africanus* were arranged in RCBD with 5 replications. The plant material was fermented in 20 L sealed containers containing 300 ml effective micro-organisms (EM), 300 ml molasses and 100 g of brown sugar. At the end of 7, 10 and 14 days of fermentation pH and Electrical Conductivity (EC) were measure. In conclusion electrical conductivity (EC) was proportional to the amount of plant material and pH inversely proportional. Therefore, the optimum quantity of plant material required for producing a bionematicide from fermented crude extracts of *Cucumis africanus* fruit was determined at 20 to 160 g in a 14-day fermentation time, yielding a pH below 3,7 at an average day/night temperature of 26ºC. In terms of plant material economy, 20 g ground fruit of *Cucumis africanus* fruit is recommended to produce a bionematicide from fermented crude extracts.

Keywords: root-knot nematodes, crude extracts, *Cucumis africanus*, fermentation, effective microorganisms.

Resumen

Se llevó a cabo un experimento de 14 días en el laboratorio de nematología de la Universidad de Limpopo para determinar la cantidad óptima de material vegetal necesaria para producir un bionematicida a partir del fruto de *Cucumis africanus* con un valor de pH inferior a 3,5. Siete tratamientos que comprenden 0, 20, 40, 80, 160, 320 e 640 g de frutos molidos de *Cucumis africanus* se dispusieron en RCBD con 5 repeticiones. El material vegetal se fermento en recipientes sellados de 20 L que contenían 300 ml de microorganismos efectivos (EM), 300 ml de melaza y 100 g de azúcar morena. Al final de los 7, 10 y 14 días de fermentación se midieron el pH y la Conductividad Eléctrica (CE). En conclusión, la conductividad eléctrica (CE) fue proporcional a la cantidad de material vegetal y el pH inversamente proporcional. Por lo tanto, la cantidad óptima de material vegetal requerida para producir un bionematicida a partir extractos crudos fermentados de frutos de *Cucumis africanus* se determinó utilizando de 20 a 160 g en un tiempo de fermentación de 14 días, obtieniendo un pH inferior a 3,7 a una temperatura promedio día/noche de 26ºC. En términos de economía de material vegetal, se recomiendan 20 g de frutos molidos de *Cucumis africanus* para producir un bionematicida a partir de extractos crudos fermentados.

Palabras clave: nematodos acalladores, extractos crudos, *Cucumis africanus*, fermentación, microorganismos efectivos.

Introduction

Root-knot nematodes continue causing severe reduction to cultivated crops globally and their management continues to be a challenge (Bali *et al*., 2018; Dutta *et al*., 2015; Rivard *et al*., 2010). Management of plant-parasitic nematodes became aggravated with continuous withdrawal of environmentally harmful nematicides, such as methyl bromide, methyl iodide and 1,3dichloropropane with proven nematicidal effect (Seid *et al*., 2015). An unexpected void was left after the withdrawal of methyl bromide, the arsenal that was relied upon for over 50 years of controlling population densities of plant-parasitic nematodes (Mashela *et al*., 2008). Prior to the withdrawal of methyl bromide from the agrochemical markets in 2005, the global annual crop losses due to plant-parasitic nematodes was estimated at USD $125 billion (Chitwood, 2003),which may be interpreted to imply that post-withdrawal estimates are much higher.

Worldwide, much work had been underway to develop non-chemical and eco-friendly approaches, such as the use of botanicals (Dutta *et al*., 2015) and organic soil amendments (Trivedi *et al*., 2017; Nagesh and Reddy, 1997) for the management of plant-parasitic nematodes. Drawbacks of conventional organic amendments have been outlined (Mashela, 2002). The ground leaching technology (GLT) system was developed for nematode suppression in smallholder farming operations in order to mitigate the drawbacks of conventional organic amendments (Mashela, 2002). Crude extracts of wild watermelon (*Cucumis africanus*) and wild cucumber (*Cucumis myriocarpus*) fruits were successfully used to suppress the southern root-knot nematode (*Meloidogyne incognita*) in tomato production (Mashela, 2002). The GLT system is labour-intensive and might be costly for use in large-scale commercial tomato producing systems. Consequently, modification of the system using fermented crude extracts of indigenous *Cucumis* fruits which could be applied through irrigation systems is increasingly essential to accommodate large commercial farming operations. The potent chemical in *Cucumis 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 *Cucumis africanus* plant (Jeffrey, 1978). Effective micro-organisms (EM) will assist in breaking the potent chemical available in *Cucumis africanus* fruit through fermentation until the pH of the solution gets below 3.5 (Kyan *el al*., 1999). The potent chemical present in *Cucumis africanus* fruit that conferred nematicidal properties had been established as 1.6 mg kg^{-1} (Mashela *et al*., 2008). In previous studies conducted by Pelinganga *et al.* (2012) fermented crude extracts of *Cucucmis africanus* fruit suppressed population numbers of*Meloidogyne incognita* race 2 and had a stimulatory effect on tomato plant growth and yield at dilution below 10%. All dilution above 10% equally suppressed nematode numbers with phytotoxic effect on the tested plant (Pelinganga *et al*., 2012). This suggests that the phytotoxicity of the plant material is proportional to quantity being use. In order to reduce or control the phytotoxicity and keep the nematicidal effect, the optimum amount of plant material must be determined. The objective of this study is to determine the optimum quantity of plant material required for producing a bionematicide from fermented crude extracts of *Cucumis africanus* fruit.

Materials and methods

The experiment was conducted at the laboratory of nematology at the Plant Protection Skills Centre, University of Limpopo, South Africa (23^{o}53"10'S, 29^{o}44"15'S) on February, 2012. Ambient day/night temperatures averaged 26^{o}C.Wild *Cucumis africanus* fruit were collected from locally cultivated plants, cut into pieces and dried in air-forced ovens at 52ºC for 72 hours

(Makkar, 1999). Dried plant material was ground in a Wiley mill to pass through a 1 mm sieve and fermented for 14 days in 20 L sealed containers having 16 L chlorine-free tapwater, 300 ml molasses, 100 g brown sugar and 300 ml EMROSA effective micro-organisms (EM) were added into each container.

Experimental design and cultural practices

Seven treatments, viz. 0, 20, 40, 80, 160, 320 and 640 g were arranged in a randomised complete block design, with 5 replicates.

Data collection

pH and EC were measured at the end of 7, 10 and 14 days of fermentation using a pH meter and an EC meter respectively.Prior to pH and EC measurements, the fermented solution was filtered.

Data analysis

Data were subjected to analysis of variance (ANOVA) through the 2010 SAS software (SASInstitute, Inc., Cary, NC., U.S.A.). Treatment mean separation was achieved using Waller-Duncan multiple range test at the probability level of 5%. Significant ($p \leq 0.05$). Unless otherwise stated; only treatments that were significant at the probability level of 5% were discussed.

Results and discussion

pH and Electrical conductivity were highly significant ($p \leq 0.000$) among the 3 measurements (7, 10 and 14 days of fermentation) carried out. All pH measurements were below 3.5 as recommended by the industry standard (Kyan *et al*., 1999), except for 0 and 640 g (7 day measurement), 640 g (10 day measurement) and 320 and 640 g (14 day measurement), (table 1).

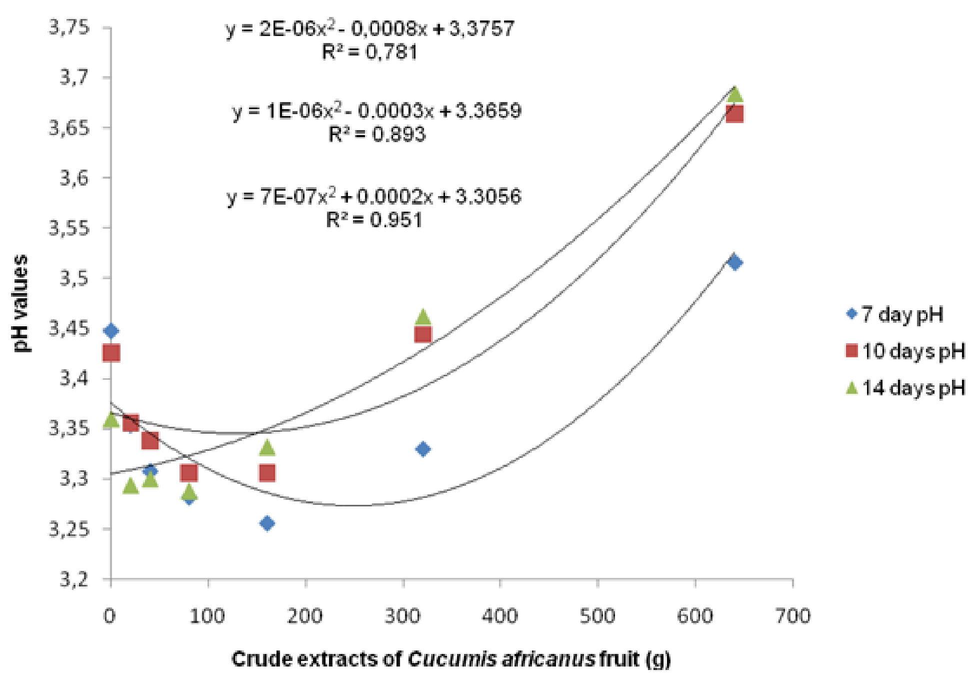
Table 1.*Cucumis africanus* PH values in 7, 10 and 14 days of fermentation.

Plant material (g)	7 days pH	10 days pH	14 days pH
0	3.448b	3.426b	3.360c
20	3.354c	3.356c	3.294d
40	3.308de	3.338cd	3.300d

80	3.282ef	3.306d	3.288d
160	3.256f	3.332cd	3.332cd
320	3.330cd	3.444b	3.462b
640	3.516a	3.664a	3.684a

The means within each column followed by the same letter were not statistically significant at probability level of 5% ($p \leq 0.05$). Source: Author´s survey.

pH values increased as time progressed from 7 to 14 days with 640 g having a pH above 3.5 throughout the experiment, whereas, 20 to 160g pH values remained statistically the same for the entire experiment. R^2 for pH measurements were 0.781, 0.893 and 0.951 respectively (Graphic 1).



Graphic 1. Influence of ground fruit of *Cucumis africanus* on pH fermented crude extracts over time (7, 10 and 14 days, respectively). Source: Author´s survey.

pH values is the major standard for fermented plant extracts readiness for use, whereas EC which can be used as an indicator of the amount of plant secondary metabolites made available through microbial fermentation has been overlooked. pH creates an environment which either suppresses or allows non-beneficial organisms to live and cause paramount problems leading to yield loss and quality crop decrease amounting to billions of dollars loss. EC unveils the amount of extracted plant material which can be rated as toxic and non-toxic to targeted and non-targeted organisms. Under ideal temperature of 26ºC as in our study 7 days are good enough to produce FCE of *Cucumis africanus* fruit yielding a pH value below 3.5. From 20 to 320 g of plant material a fermented solution with a pH value below 3.5 was produced. As the days progressed to 10 and 14 respectively, there was a tendency to increase the pH value at all levels with major increases in the range of 320–640 g. Plant material ranging from 320–640 g would require more fermentation time in terms of days to produce a pH value below 3.5. On the other hand the toxicity of the plant material might have hindered microbial activity (320–640g) in lowering the pH of the solution to below 3.5. The poisonous form of the fruit contains the bitter principle *cucumin* which is extremely bitter and very toxic (Van Wyk *et al*., 2002), the bitterness of the plant material increases with increase in the quantity of plant material probably making the medium unsuitable for microbial activity. A high pH value above 3.5 is a sign that the fermented solution is not ready for use (Kyan *et al*., 1999). Considering fermentation time, 7 days is economically feasible in terms of time and product delivery for use.

The amount of plant material to be used plays an important role as the industry always strives at getting an optimum output with less input. The plant material ranging from 20–160 g was not statistically different from the control and yielded pH values within the acceptable industry set standard, unlike 320–640 g which did not generated pH values below 3.5. This means that any amount of plant material ranging from 20–160g can be used to produce a FPE of *Cucumis africanus* fruit resulting in a solution with a pH value below 3.5. However, in terms of economics, the lowest quantity of plant material would be ideal. Therefore, 20 g should be used to produce FCE of *Cucumis africanus* fruit for use in plant protection against root-knot nematodes.

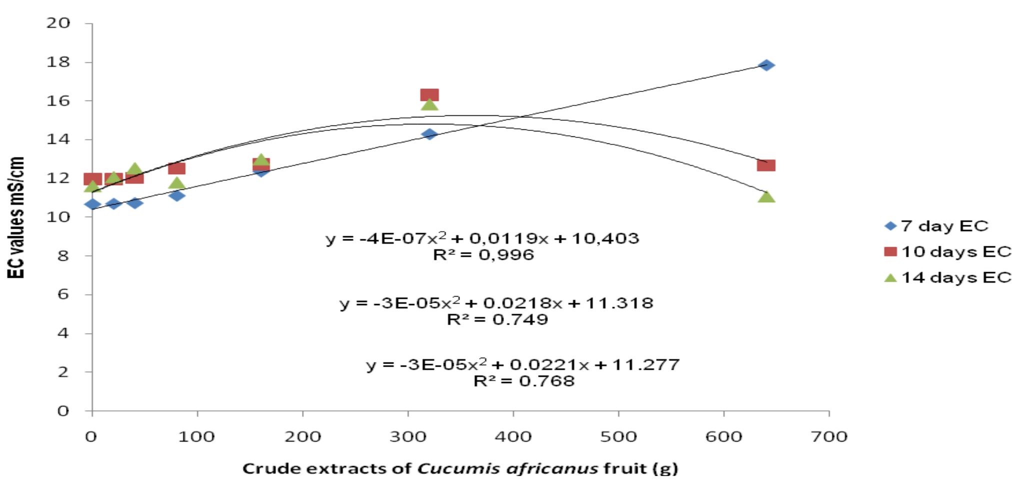
EC was proportional to the amount of plant material in all measurements except for 640 g (10 and 14 day measurement) showing some fluctuations, (table 2).

Table 2.*Cucumis africanus* EC values in 7, 10 and 14 days of fermentation.

Plant material (g)	7 day EC	10 days EC	14 days EC
0	10.654d	11.948b	11.616bc
20	10.676d	11.962b	12.074bc
40	10.712d	12.024b	12.532bc
80	11.094d	12.496b	11.798bc
160	12.348c	12.716b	12.990b
320	14.274b	16.332a	15.814a
640	17.830a	12.656b	11.084c

The means within each column followed by the same letter were not statistically significant at probability level of 5% ($p \leq 0.05$). Source: Author´s survey.

EC values increased from 20 to 640 g, thus, more secondary metabolites were mined from high volume of plant material. However, EC is not used as the indicator that the fermented solution is ready for use, but rather the pH value below 3.5 (Kyan *el al*., 1999) R^2 for EC measurements were 0.996, 0.749 and 0.768 respectively (Figure 2).



Graphic 2. Influence of ground fruit of *Cucumis africanus* on Electrical conductivity of fermented crude extracts over time (7, 10 and 14 days, respectively). Source: Author´s survey.

The EC value increased with the amount of plant material. In this study was observed higher EC values as the amount of plant material increased from 20–640 g. The real amount of plant material EC value is obtained from subtracting the EC value from the control which has no plant material, the result is the actual plant material (secondary metabolites) made available through fermentation. For example, 10,676 – 10,654 = 0,022; then (20 – 0 g = 0,022) and 0,022 is what the micro-organisms made available from 20 g of plant material, since cucurbitacin B ($C_{32}H_{48}O_8$), the potent chemical identified in *Cucumis africanus* fruit is insoluble in water (Chen *et al*., 2005; Jeffrey, 1978). The highest EC values are obtained as the plant material is increased in grams.

Conclusion

Seven days are required to produce FCE of *Cucumis africanus* fruit under a temperature of 26ºC. 20 – 160g of plant material is adequate to produce a fermented solution with a pH value below 3.5. However, in term of economics 20 g should be used since from 20 – 160 g are statistically equal. A greenhouse, microplot and field experiments were conducted using a fermented solution from 20 g, which proved to be effective in the control of *Meloidogyne incognita* race 2 without being phytotoxic to tomato plants (Pelinganga *et al*., 2012).

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