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# Optimum Harvest Time of *Cucumis africanus* Fruit Using Concentration of Cucurbitacin B as a Maturity Standard

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**Abstract**

Fruit of wild watermelon (*Cucumis africanus*) are used to manufacture nemafric-BL phytonematicide for the management of population densities of the notorious root-knot (*Meloidogyne* species) nematodes on various crops. However, the concentration of the active ingredient, namely, cucurbitacin B, is dependent on the developmental stage of the fruit. A field study was, therefore, conducted to establish the optimum harvest time where *C. africanus* fruit would have the highest concentration of cucurbitacin B in order to ensure consistent quality of the phytonematicide. Fruit were harvested weekly from 60 to 110 days after transplating, prepared for extraction and cucurbitacin B quantified using reverse phase HPLC. Concentrations of cucurbitacin B were optimised at 5.0 weeks, which translated to a 95-day harvest time after seeding.

**Keywords:** allelochemicals, cucurbitacins, density-dependent growth, phytonematicides, secondary metabolites

## 1. Introduction

### *1.1 The Research Problem*

Maturity tests are inherently used in various crop industries to establish the optimum harvest time (Lee et al., 1983; Saevels et al., 2003; Lebrun et al., 2004, 2008). This ensures consistent quality of the produce (ISO 9000, 2000), and the quality of the products (Parasuraman et al., 1990). Generally, maturity tests could include the quantification of total soluble solids (TSS), total acidity, pH, acid/sugar ratio, tannins, volatile compounds, ascorbic acid and oil content (Abbasi et al., 2011; El-Buluk et al., 1995; Hulme, 1971; Kader, 2002). Most established maturity tests require some form of equipment or laboratory procedure (Wilson, 2013; Wilson & Baietto, 2009) and could not always be accessible to developing industries. Generally, maturity tests should not be confounded with maturity indices, which comprise the use of various shapes and colours during grading of various produce (Cristo, 1994). The introduction of alternative crops is confronted with various challenges, amongst which could be to ensure consistent quality of the product, which is associated with maturity testing.

### *1.2 Importance of Research Problem*

Wild watermelon (*Cucumis africanus* L. F.), indigenous to Limpopo Province, South Africa (Kristkova et al., 2003), has widely been used in various industries, which range from nutraceutical, pharmaceutical, cosmeceutical and pesticidal industries (Lee et al., 2010; Mashela et al., 2011; Thies et al., 2010; Van Wyk & Wink, 2012; Van Wyk et al., 2002). The active ingredient had been identified as cucurbitacin B (Chen et al., 2005), which is an allelochemical compound ($C_{32}H_{46}O_8$) that is distributed equally in all organs of *C. africanus*. Nemafric-BL phytonematicide has been researched and developed from *C. africanus* fruit under greenhouse, microplot and field conditions (Mashela et al., 2011). This phytonematicide has the attributes of serving as an alternative to methyl bromide, which had since been withdrawn from the agro-chemical markets due to its environment-unfriendliness (Mashela et al., 2011).

*1.3 Relevant Scholarship*

Biological entities, including plant-parasitic nematodes, respond to increasing concentrations of allelochemicals in density-dependent growth (DDG) patterns (Pelinganga et al., 2012; Salisbury & Ross, 1992). Generally, the DDG patterns have three phases, *viz*; stimulation, neutral and inhibition phases (Liu et al., 2003; Pelinganga et al., 2012; Salisbury & Ross, 1992). In most cases, DDG patterns are characterized by quadratic curves (Pofu & Mashela, 2014; Salisbury & Ross, 1992), which allow for the computation of the optimum point in the neutral phase (Pofu & Mashela, 2014). Generally, the optimum point is the independent (x-axis) variable for the highest level of the dependent (y-axis) variable. Pelinganga et al. (2012) used the DDG patterns to establish the application interval of nemafric-BL 3% phytonematicide as 18 days for effective suppression of root-knot (*Meloidogyne* species) nematode population densities in tomato (*Solanum lycopersicum* L.) production.

*1.4 Hypothesis and Its Correspondence to Research Design*

Cucurbitacins, as secondary metabolites, accumulate in various organs of plants within the Cucurbitaceae Family (Chen et al., 2005). The accumulation of cucurbitacins in organs over time could probably follow the DDG patterns during various developmental stages of plants, which would allow for the determination of the optimum time for the highest accumulation of cucurbitacins. The objective of this study, therefore, was to use the accumulation of cucurbitacin B during different developmental stages of fruit in *C. africanus* to establish the optimum harvest time in order to ensure consistent quality of nemafric-BL phytonematicide.

## 2. Materials and Methods

*2.1 Study Field and Treatments*

The field study was conducted at the Green Technologies Research Centre, University of Limpopo, Limpopo Province, South Africa (23°53′10″S, 29°44′15″E). Seeds were prepared as described previously (Mafeo & Mashela, 2009) and sown in seedling trays containing Hygromix (Hygrotech, Pretoria North, South Africa) growing medium. At two-leaf stage, seedlings were hardened off for 5 days, selected for uniformity and transplanted at 4 weeks. Plot size was 1 m x 1 m, each containing 4 seedlings. Treatments, viz., 60, 67, 74, 81, 89, 96, 103 and 110 harvest days after transplanting, were arranged in randomised complete block design, with 12 replications. 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, with irrigation being every other day.

*2.2 Preparation, Extraction and Quantifying Cucurbitacin B*

Eight fruit from each replication of *C. africanus* were harvested weekly, cut into pieces, dried at 52 °C for 72 h in an air-forced oven and ground in a Wiley mill to pass through a 1-mm-pore sieve (Makkar, 1991). Four grams of dried crude extracts of fruit were mixed with 50:50 ml methanol and dichloromethane and allowed to run for 4 h on a waterbath at 40 °C at 45 rpm (Rotary Evaporator). After extraction, sub-samples were concentrated by reducing the volume to 30 ml and then 1 ml centrifuged at 4500 rpm for 10 minutes before filtering through 0.22 µm-pore filter (Miller, Sigma). A cucurbitacin B reference standard obtained from Wuhan ChemFaces Biochemical Co., Ltd. (Wuhan, China), was dissolved in methanol, with a range of standard concentrations (0.01-1.0 mg/ml) prepared.Concentration of cucurbitacin B was quantified using a Shimadzu Prominence HPLC system (Shimadzu Corporation, Kyoto, Japan) equipped with a Discovery® Wide Pore C18 reverse phase column (25 cm × 4.0 mm) with 5 µm particle size packing. Isocratic elution was done using methanol/water (40:60) (v/v) at flow rate of 1 ml/min. Eluents were detected using a Shimadzu CTO-20A photo diode array detector (PDA) and the peaks were quantified using Shimadzu LC solutions software.

*2.3 Data Analysis*

Cucurbitacin B data were subjected to analysis of variance (ANOVA) procedure using SAS software (SAS Institute Inc., 2008). When the treatments were significant at the probability level of 5%, the degrees of freedom and their associated sum of squares were partitioned to determine the percentage contribution of sources of variation to the total treatment variation (TTV) among the treatment means (Steyn et al., 2003). Mean separation was achieved using Waller-Duncan multiple range test. The variable with significant ($P \leq 0.01$) treatment means were further subjected to lines of the best fit using cucurbitacin B responses to different harvest time and modelled by the regression curve estimations resulting in a quadratic equation: $Y = b_2x^2 + b_1x + a$, where Y = Cucurbitacin B concentration and x = harvest time with $-b_1/2b_2 = x$ being the value for the optimum cucurbitacin B concentration. Unless otherwise stated, only treatment means significant at the probability level of 5% were discussed.

## 3. Results

Harvest time had highly significant ($P \leq 0.01$) effects on concentration of cucurbitacin B in *C. africanus* fruit and contributed 73% in total treatment variation of cucurbitacin B concentration. Cucurbitacin B (y-axis) and harvest time (x-axis) had quadratic relationships, where the model explained 87% of the observed variation in cucurbitacin B (Figure 1). The concentration of cucurbitacin B was optimised at 5.0 weeks starting from 60 days after transplanting four-week old seedlings (Table 1), which translated to harvest time of approximately 95 days after seeding. Relative to initial harvest time (60 days), all other harvest times had strong impact to the accumulation of cucurbitacin B concentration as shown by relative increases ranging from 22% to 898% (Table 2).

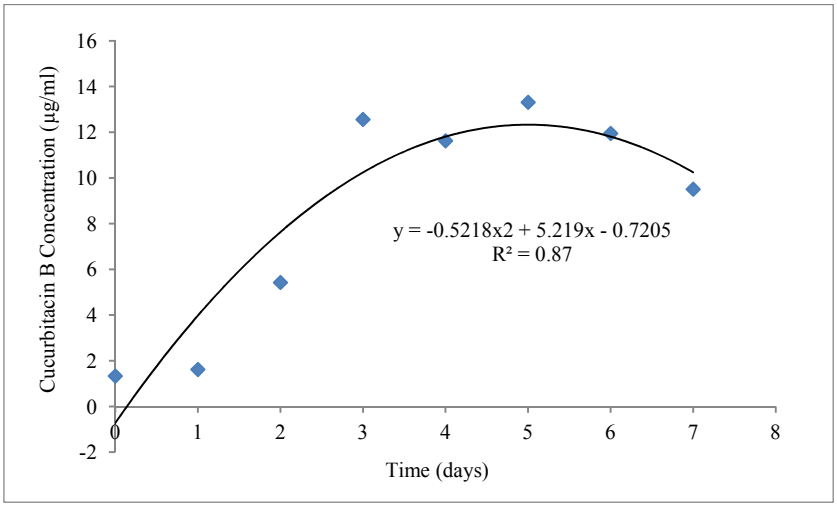


Figure 1. Responses of cucurbitacin B concentration from *Cucumis africanus* overtime ($T_0$ = 60 DAT) (n= 96).

Table 1. Quadratic relationship, coefficient of determination and computed optimum harvesting time of *Cucumis africanus* fruit (n = 96)

| Plant variable | Quadratic equation | $R^2$ | x | P ≤ |
|---|---|---|---|---|
| CuB concentration | $Y = -0.5218x^2 + 5.219x - 0.7205$ | 87 | 5.0 | 0.00 |
| Optimum harvesting time (weeks) 5.0 | | | | |

*Note*. Calculated optimum harvesting time (x) = $-b_1/2b_2$, where for CuB $b_1 = 5.219$ and $b_2 = -0.5218$.

Table 2. Relative accumulation of cucurbitacin B in fruit of *Cucumis africanus* over increasing developmental stages of fruit (n = 96)

| Treatment | Y-value | Relative impact$^z$ (%) |
|---|---|---|
| 0 | 1.332 | - |
| 1 | 1.619 | 22 |
| 2 | 5.423 | 307 |
| 3 | 12.558 | 843 |
| 4 | 11.623 | 773 |
| 5 | 13.306 | 898 |
| 6 | 11.946 | 796 |
| 7 | 9.5056 | 613 |

*Note*. Impact$^z$ = treatment/control (0) – 1) × 100.

## 4. Discussion

Generally, there are five developmental stages during the formation of fruits in *Cucumis* species (Shaik et al., 2011). Flowering is followed by fruit formation, which is characterised by rapid cell division of the ovary. Elongation stage, which proceeds for two to three weeks, ensues. Thereafter, fruit maturation stage occurs, characterised by fruit swelling without increases in fruit size. Colour changes from green to slightly yellow signify fruit ripening. Finally, senescence sets in, resulting in softening and thereafter, fruit shriveling (Oh et al., 2011; Shaik et al., 2011). Consequently, results of the study suggested that the optimum concentration of cucurbitacin B coincided with the maturity stage in *C. africanus* fruit as observed in other maturity standards (Abbasi et al., 2011; El-Buluk et al., 1995; Gry et al., 2006; Hulme, 1971; Kader, 2002).

The response of cucurbitacin B level to varying fruit developmental stages demonstrated a DDG pattern (Liu et al., 2003). Biological entities respond to extrinsic and intrinsic factors in a DDG pattern (Liu et al., 2003). Declining and increasing levels of cucurbitacin B concentration coincided with senescence and maturity stages, respectively. Results of this study agree with the finding of cucurbitacin concentration in *Ecballium elaterium* and *Trichosanthes cucumerina* L. var. *cucumerina,* where the concentration of cucurbitacin reached the highest peak during fruit maturation stage (Attard & Scicluna-Spiteri, 2004; Devendra et al., 2011).

The production of allelochemicals is highly dependent on the physiological and developmental stage of plants. Developmental stages have an impact on the metabolic pathways which are responsible for accumulation of secondary metabolite (Achackzai et al., 2009; Devendra et al., 2011; Ramakrishna & Ravishankar, 2011). The observed decline in the concentration of cucurbitacin B during senescence in our study was an important observation since this could also have a detrimental effect on quality of nemafric-BL. However, the mechanism involved in this decline is not yet clear, but could be suggesting the existence of reversible reactions as shown in the accumulation of other allelochemical compounds (Jilani et al., 2008).

## 5. Conclusion

In conclusion, results suggested that concentration of cucurbitacin B in *C. africanus* was optimised at maturation phase, which was followed by decline in the concentration of this allelochemical. Consequently, accurate timing of the harvest time could be essential in ensuring consistent quality of nemafric-BL.

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## References

Abbasi, K. S., Anjum, N., Sammi, S., Masud, T., & Ali, S. (2011). Effect of coatings and packaging material on the keeping quality of mangoes (*Mangifera indica* L.) stored at low temperature. *Pakistan Journal of Nutrition, 10*, 129-138. http://dx.doi.org/ 10.3923/pjn.2011.129.138

Achackzai, A. K. K., Achakzai, P., Masood, A., Kayani, S. A., & Tareen, R. B. (2009). Response of plant parts and age on the distribution of secondary metabolites on plants found in Quetta. *Pakistan Journal of Botany, 41*, 2129-2135.

Attard, E., & Scicluna-Spiteri, A. (2004). The cultivation and cucurbitacin content of *Ecballium elaterium* (L.) A. rich. *Cucurbit Genetics Cooperative Report, 27*, 73-76.

Chen, J. C., Chiu, M. H., Nie, R. L., Cordell, G. A., & Qiu, S. X. (2005). The cucurbitacins and cucurbitane glycosides: Structures and biological activities. *Natural Product Report, 22*, 386-399. http://dx.doi.org/10.1039/b418841c

Cristo, C. H. (1994). Stone fruit maturity indices: a descriptive review. *Posharvest News Information, 5*, 65-68.

Devendra, N. K., Attard, E. G., Raghunandan, D., & Seetharam, Y. N. (2011). Study of seasonal variation on the content of cucurbitacin of various parts of *Trichosanthes cucumerina* L. var. cucumerina. *International Journal of Plant Research, 1*, 25-28. http://dx.doi.org/10.5923/j.plant.20110101.04

El-Buluk, R. E., Babikar, E. E., & El Tinay, A. H. (1995). Biochemical and physical changes in fruits of four guava cultivars during growth and development. *Food Chemistry, 54*, 279-282. http://dx.doi.org/10.1016/0308-8146(95)00047-M

Gry, J., Søborg, I., & Andersson, H. C. (2006). *Cucurbitacins in plant food*. Copenhagen, Denmark: Ekspressen Tryk and Kopicenter.

Hulme, A. C. (1971). The Mango. In A. C. Hulme (Ed.), *The biochemistry of fruits and their products*. London &

New York: Academic Press.

ISO 9000. (2000). *Quality management systems – fundamental and vocabulary*. Switzerland: International Organisation for Standardisation.

Jilani, G., Mahmood, S., Chaudhry, A. N., Hassan, I., & Akram, M. (2008). Allelochemicals: sources, toxicity and microbial transformation in soil - A Review. *Annals of Microbiology, 58*, 351-357. http://dx.doi.org/10.1007/BF03175528

Kader, A. A. (2002). Standardization and Inspection of Fresh Fruits and Vegetables. In A. A. Kader (Ed.), *Postharvest Technology of Horticultural Crops* (pp. 287-300). Oakland, CA: University of California.

Kristkova, E., Lebeda, A., Vinter, V., & Blahousek, O. (2003). Genetic resources of genus *Cucumis* and their morphological description (English-Czech Version). *HortScience, 30*, 14-42.

Lebrun, M., Ducamp, M. N., Plotto, A., Goodner, D., & Baldwin, E. (2004). Development of electronic nose measurements for mango (*Mangifera indica*) homogenate and whole fruit. *Proceeding of the Florida State Horticultural Society, 117*, 421-425.

Lebrun, M., Plotto, A., Goodner, K., Ducamp, M., & Baldwin, E. (2008). Discrimination of mango fruit maturity by volatiles using the electronic nose and gas chromatography. *Postharvest Biology and Technology, 48*, 122-131.

Lee, D. H., Iwanski, G. B., & Thoennissen, N. H. (2010). Cucurbitacin: Ancient compound shedding new light on cancer treatment. *Scientifc World Journal, 10*, 413-418. http://dx.doi.org/10.1100/tsw.2010.44

Lee, S. K., Young, R. E., Schifman, P. M., & Coggins, C. W. Jr. (1983). Maturity studies of avocado fruit based on picking dates and dry weight. *Journal of the American Society Horticultural Science, 108*, 390-394.

Liu, D. L., An, M., Johnson, I. R., & Lovett, J. V. (2003). Mathematical modeling of allellopathy. III. A model for curve-fitting allellochemical dose responses. *Non-linearity in Biology, Toxicology and Medicine, 1*, 37-50. http://dx.doi.org/10.1080/15401420390844456

Mafeo, T. P., & Mashela, P. W. (2009). Responses of germination in tomato, watermelon and butternut squash to a *Cucumis* bio-nematicide. *Journal of Agriculture and Environmental Science, 6*, 215-219.

Makkar, H. P. S. (1991). *Quantification of Tannis in Tree Foliage*. IAEA Working Document. Viena, Australia: IAEA.

Mashela, P. W., De Waele, D., & Pofu, K. M. (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. *Scientific Research and Essays, 6*, 6762-6768.

Oh, S. H., Lim, B. S., Hong, S. J., & Lee, S. K. (2011). Aroma volatile changes of netted muskmelon (*Cucumis melo* L.) fruit during developmental stages. *Horticulture, Environment and Biotechnology, 52*, 590-595.

Parasuraman, A., Berry, L. L., & Zeithaml, V. A. (1990). *An empirical examination of relationship in an extend service quality model*. Cambridge, MA: Marketing Science Institute.

Pelinganga, O. M., Mashela, P. W., Nzanza, B., & Mphosi, M. S. (2012). Baseline information on using fermented crude extracts from *Cucumis africanus* fruit for suppression of *Meloidogyne incognita* and improving growth of tomato plant. *African Journal of Biotechnology, 11*, 11407-11413.

Pofu, K. M., & Mashela, P. W. (2014). Density-dependent growth patterns of *Meloidogyne javanica* on hemp cultivars: Establishing nematode-sampling timeframes in host-status trials. *American Journal of Experimental Agriculture, 4*, 639-650.

Ramakrishna, A., & Ravishankar, G. A. (2011). Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signaling and Behavior, 6*, 1720-1731. http://dx.doi.org/10.4161/psb.6.11.17613

Saevels, S., Lammertyn, J., Berna, A. Z., Veraverbeke, E. A., Di Natale, C., & Nicolai, B. M. (2003). Electronic nose as a non-destructive tool to evaluate the optimal harvest date of apples. *Postharvest Biology and Technology, 30*, 3-14.

Salisbury, F. B., & Ross, C. W. (1992). *Plant physiology* (4th ed.). Belmont (CA): Wadsworth.

SAS Institute Inc. (2008). *SAS/STAT® 9.2 qualification tool users's guide*. Carry (NC), USA: SAS Inc.

Shaik, R. S., Weston, L. A., Burrows, G., & Gopurenko. D. A. (2011). *Comparative phenological and genetic*

*diversity analysis of two invasive weeds, camel melon (Citrullus lanatus (Thunb.) matsum. and nakai var. lanatus) and prickly paddy melon (Cucumis myriocarpus L.) in inland Australia*. 23rd Asian-Pacific Weed Science Society Conference. Australia: The Sebel Cairns.

Steyn, A. G. W., Smit, C. F., DU Toit, S. H. C., & Strasheim, C. (2003). *Modern statistics in practice*. Pretoria, South Africa: Van Schaik.

Thies, J. A., Ariss, J. J., Hassell, R. L., Olson, S., Kousik, C. S., & Levi, A. (2010). Grafting for management of southern root-knot nematode, *Meloidogyne incognita*, in watermelon. *Plant Disease, 94*, 1195-1199.

Van Wyk, B. E., & Wink, M. (2012). *Medicinal Plants of the World: An Illustrated Scientific Guide to Important Medicinal Plants and Their Uses*. Pretoria, South Africa: Briza Publications.

Van Wyk, B., Heerden, F., & Outshoorn, B. (2002). *Poisonous Plants of South Africa*. Pretoria, South Africa: Briza Publications.

Wilson, A. D. (2013). Diverse applications of electronic-nose technologies in agriculture and forestry. *Sensors, 13*, 2295-2348. http://dx.doi.org/ 10.3390/s130202295

Wilson, A. D., & Baietto, M. (2009). Applications and advances in electronic-nose technologies. *Sensors, 9*, 5099-5148. http://dx.doi.org/ 10.3390/s90705099