

Full Length Research Paper

Study of chemical residues from Nemarioc-AL and Nemafric-BL phytonematicides in tomato fruit

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Pesticide chemical residues in produce and products are a global concern in human and animal diets. Nemarioc-AL (a.i. cucurbitacin A) and Nemafric-BL (a.i. cucurbitacin B) phytonematicides serve as alternatives to synthetic nematicides in the management of root-knot nematodes (*Meloidogyne* spp.) in tomato (*Solanum lycopersicum*) production. Cucurbitacins are the bitterest chemical compounds, and therefore, could affect fruit taste when used in tomato production to manage nematodes. The objective of this study was to determine whether tomato fruit where nematode numbers were managed using the two phytonematicides, contain cucurbitacin chemical residues. A field study using tomato cv. 'Rodade' was initiated, with untreated control, Nemarioc-AL and Nemafric-BL phytonematicides, laid out in randomised complete block design, with thirteen replications. Each phytonematicide at 3% was applied at a 17-day interval. At 110 days, after initiating the treatments, fruit were sampled and analysed for chemical residues using the isocratic elution Shimadzu HPLC Prominence with Shimadzu CTO-20A diode array detector. Cucurbitacin A and B residues were not detected in 'Rodade' fruit and therefore, the two phytonematicides were suitable for nematode management in tomato production under field conditions.

Key words: Botanical, botinemagation, *Cucumis myriocarpus*, *Cucumis africanus*, nematodes.

INTRODUCTION

Nemarioc-AL (L = liquid formulation) and Nemafric-BL phytonematicides, used through botinemagation (Mashela et al., 2015), are highly effective on suppression of nematode population densities (Pelinganga and Mashela, 2012; Pelinganga et al., 2012; 2013). Efficacy trials using any of the two products had been conducted against root-

knot nematodes (*Meloidogyne* spp.) and the citrus nematode (*Tylenchulus semipenetrans*) under diverse conditions (Mashela et al., 2015). The two products are produced from fermented crude extracts of fruits collected from wild cucumber (*Cucumis myriocarpus*) and wild watermelon (*Cucumis africanus*), respectively, which

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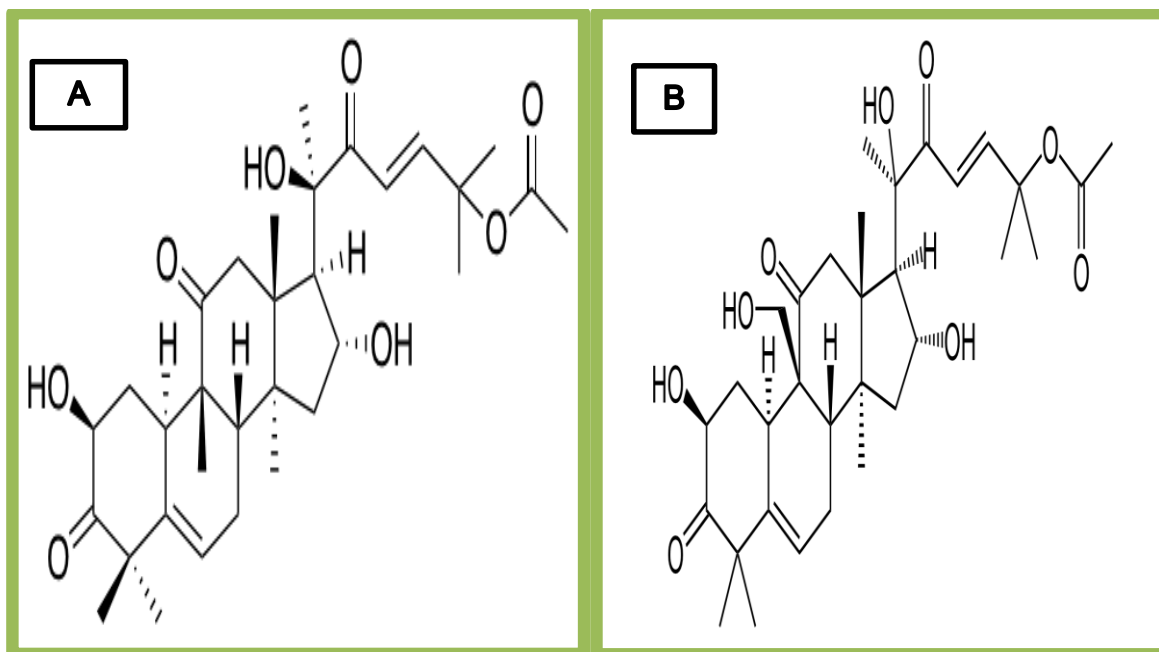


Figure 1. The chemical structure of cucurbitacin A ($C_{32}H_{46}O_9$) (A) and cucurbitacin B ($C_{32}H_{46}O_8$) (B) (PubChem, Open chemistry database).

are indigenous to Limpopo Province, South Africa (Kristkova et al., 2003). The bioactive chemical compounds in fruits of the two *Cucumis* species are cucurbitacin A and B (Figure 1), respectively.

In efficacy trials of phytonematicides, care should be taken to ensure that (a) phytotoxicity is avoided, (b) the product consistently suppresses nematodes and (c) the product does not result in chemical residues in produce, thereby, affecting produce quality (Mashela et al., 2015). Most phytotoxicity and consistency trials for the two products had been completed (Mashela et al., 2015), with limited information on chemical residues in produce.

The objective of this study was to determine the potential existence of chemical residues in fruit of tomato (*Solanum lycopersicum*) plants where nematode population densities were managed using Nemarioc-AL and Nemafric-BL phytonematicides.

MATERIALS AND METHODS

Study location

A field study was conducted at the Green Technologies Research Centre, University of Limpopo, South Africa (23°53'10"S, 29°44'15"E). The site has mean annual rainfall of less than 500 mm and minimum/maximum average temperatures of 10 /38°C. The predominant soil form are Hutton [65% sand, 30% clay, 5% silt; 1.6% organic C, EC 0.148 dS/m and pH(H₂O) 6.5]. The experiments were conducted in summer (October – December) 2015 with high initial nematode population densities ($P_i = 3000$ J2 *Meloidogyne* species) and repeated in autumn (January – March) 2016 on an adjacent field with P_i averaging 3500 J2 *Meloidogyne*

species.

Plant materials and cultural practices

Fruits of the two *Cucumis* species were collected from cultivated fields, separately cut into pieces, dried at 52°C and milled in a Wiley mill as previously described (Shadung et al., 2015). The products were prepared using standard fermentation procedures (Mashela et al., 2015). Uniform four week-old nematode-susceptible tomato cv. 'Rodade' seedlings were transplanted in a field with intra- and inter-row spacing of 0.5 and 1.0 m, respectively. Three days after transplanting, each seedling was fertilised with 3 g 2:3:2 (22) NPK fertiliser mixture/plant to provide 186 mg N, 126 mg K and 156 mg P per mL water and 2 g 2:1:2 (43) Multifeed to provide 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. Plants were irrigated using a drip irrigation system that discharged 2 L/h as recommended for commercial tomato production in the region. Plants were scouted daily for insect pests which were managed using pesticides recommended for tomato production when numbers were higher than 10. Plants were sprayed weekly using Dithane M-45 which was alternated with Bravo and copper oxychloride for disease management.

Experimental design and treatments

The three treatments, untreated control, Nemafric-BL and Nemarioc-AL phytonematicides, were arranged in a randomised complete block design, with seven and six replications in 2015 and 2016, respectively. Treatments were initiated a week after transplanting. Irrigation was substituted for treatments using 3% concentration of each phytonematicide applied at 17-day interval (Mashela et al., 2015). Phytonematicides were drench-applied around the root systems using 1000 mL solution/plant.

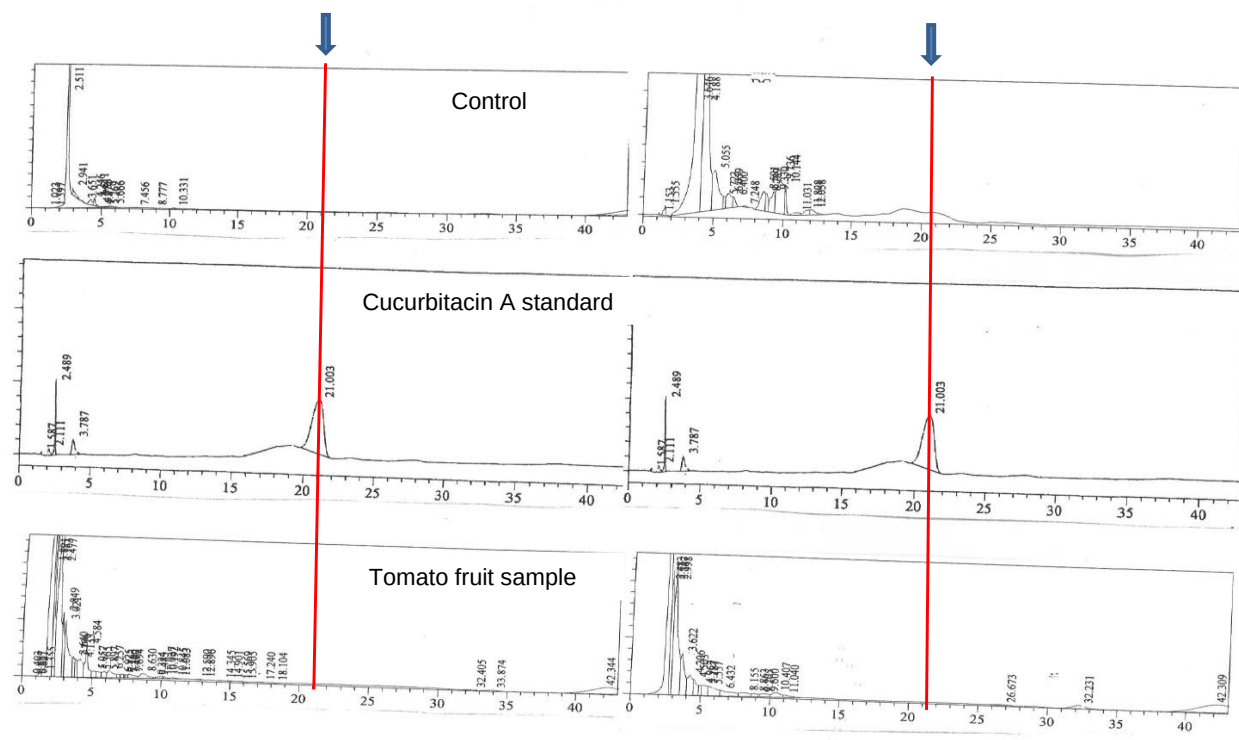


Figure 2. Chromatograms for untreated control, cucurbitacin A standard and tomato fruit samples where plants were treated with Nemarioc-AL phytonematicide.

Extraction, quantifying cucurbitacin

At 110 days after initiating the treatments, with the withholding period of 15 days, tomato fruit were collected, cut into pieces and oven-dried at 52°C for 72 h and milled. Four grams subsample of milled tomato fruit/treatment was extracted in 100 mL methanol and dichloromethane ($\geq 99.8\%$) at 1:1 (v/v) solution on a rotary evaporator (Buchi Labortechnik, Essen, Germany) set at 60 rpm at 40°C for 4 h. After extraction, subsamples were concentrated by reducing the volume from 100 to 30 mL under reduced pressure on a rotary evaporator and then 1 mL aliquot of each treatment centrifuged at 4500 rpm for 10 min before filtering through 0.22 μ m-pore filter (Miller, Sigma, South Africa).

Cucurbitacin A and B were each tested with the isocratic elution Shimadzu HPLC Prominence (Kyoto, Japan) with Shimadzu CTO-20A diode array detector. A wide pore reverse phase C18 (25 cm $\times$ 4.0 mm, 5 μ m) discovery (Sigma-Aldrich, Milan, Italy) with 2:3 (v/v) methanol and deionised water as a mobile phase at a flow rate of 1.0 mL/min in an oven at 35°C and the wavelengths monitored at 230 nm for 43 min were performed to quantify the cucurbitacins. Pure ($\approx 98\%$) cucurbitacin A and B standards (Wuhan ChemFaces Biochemical Co. Ltd., Wuhan, China) were each dissolved in methanol and then diluted at 0.02, 0.04, 0.06, 0.08 and 1.0 μ g/mL to compare the retention times and the peak areas of standards and samples.

RESULTS AND DISCUSSION

In plants treated with Nemarioc-AL phytonematicide, the retention peak for cucurbitacin A standard was achieved at ca. 21 min, whereas both untreated control and treated

tomato fruit samples did not have retention peaks (Figure 2). Similarly, in plants treated with Nemafric-BL phytonematicide, the retention peak for cucurbitacin B standard was achieved at 35.257 min, whereas the untreated control and treated tomato fruit samples had no retention peaks (Figure 3). In short, cucurbitacin chemical residues for both products were not observed in tomato fruit.

Cucurbitacin A ($C_{32}H_{46}O_9$) and cucurbitacin B ($C_{32}H_{48}O_8$) have different large chemical structures, with cucurbitacin A being partially polar and slightly soluble in water (Chen et al., 2005), whereas cucurbitacin B is non-polar and insoluble in water (Jeffrey, 1978). Non-polar molecules such as glucose cannot be transported through the bipolar membranes of the symplastic pathways in the endodermis of roots into the vascular bundle (Campbell, 1990). This could explain why cucurbitacin chemicals were not detected in tomato fruit samples in this study (Figure 2 and 3). The withholding period of 15 days in the current study could have also contributed to the observed results. In the 1 to 7-day withholding period, azadirachtin ($C_{35}H_{44}O_{16}$) residues were detected in olive (*Olea europaea*) but the residues declined rapidly from 0.35 ppm in Day-1 to less than 0.02 ppm in Day-7 after application (Caboni et al., 2002). In contrast, when using neem products on strawberry (*Fragaria ananassa*), azadirachtin chemical residues in berries were not detected at 7 days after application

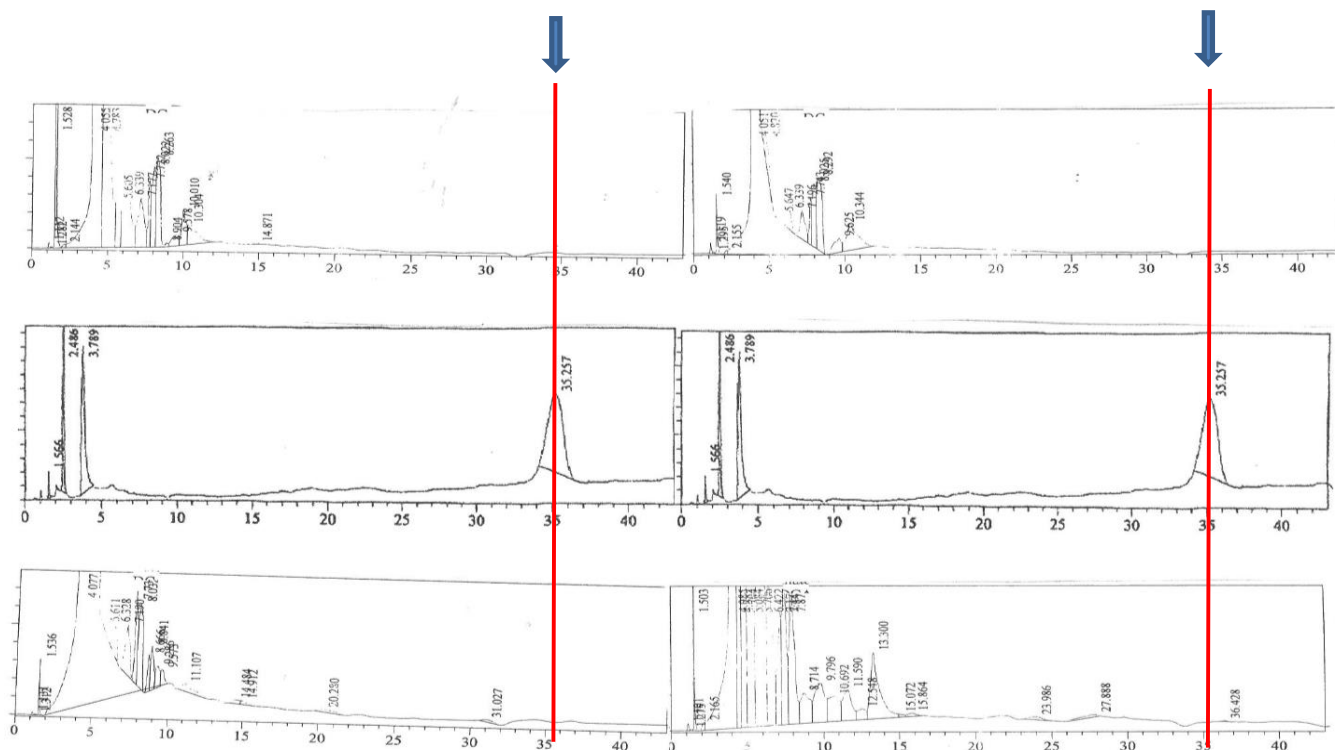


Figure 3. Chromatograms for untreated control, cucurbitacin B standard and tomato fruit samples where plants were treated with Nemafric-BL phytonematicide.

(Caboni et al., 2006). The azadirachtin findings along with the undetected concentration of cucurbitacins at 15-day withholding period in tomato fruit (Figures 2 and 3), suggested that these products could be “viewed” as being “safe” for human consumption in produce where nematode population densities were suppressed using the products.

In density-dependent growth (DDG) context (Mashela et al., 2015), the accumulation of cucurbitacin in edible produce and in subsequent products would be risky since in small quantities that characterise chemical residues, cucurbitacins could stimulate cell division in animals, thereby inducing cancer (Lee et al., 2010). However, at high concentrations, where growth of cells is inhibited, the cucurbitacins would be cytotoxic (Lee et al., 2010). The observation in this study, where cucurbitacins were undetectable (Figures 2 and 3), is important for the potential commercialisation of Nemarioc-AL and Nemafric-BL phytonematicides.

Conclusion

At 3%, 17-day application interval and 15-day withholding period, Nemarioc-AL and Nemafric-BL phytonematicides had no traceable cucurbitacin residues in fruit samples of tomato cv. ‘Rodade’. Further studies would, however, be

necessary to assess the potential cucurbitacin residues within shorter withholding periods.

CONFLICT OF INTEREST

The authors have not declared any conflict of interest.

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