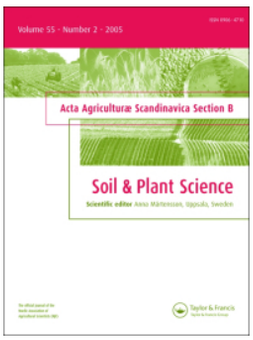

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Comparative efficacy of cucurbitacin phytonematicides and Velum on growth and fruit quality of watermelon cultivar 'Congo' and suppression of *Meloidogyne enterolobii* under field conditions

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ABSTRACT

Globally, the guava root-knot nematode (*Meloidogyne enterolobii*) is becoming an emerging threat of note in crops with or without Mi resistance genes. Watermelon (*Citrullus lanatus*) cultivars are highly susceptible to *Meloidogyne* species, with all cultivars without genotypes with resistance to the genus. In contrast, nematode management options for watermelon production had since the withdrawal of fumigant nematicides been constrained. The objective of this study was to investigate the comparative efficacy of the locally-developed cucurbitacin phytonematicides and commercially available synthetic chemical nematicide Velum on growth and fruit yield and quality of watermelon cv. 'Congo', along with its accumulation of foliar nutrient elements and suppression of *M. enterolobii* population densities under field conditions. Nemarioc-AL and Nemafric-BL phytonematicides were each applied biweekly at 2% per seedling using 500 ml solution, while Velum was applied once using 500 ml solution at 0.08 ml/15 L chlorine-free water. At 90 days after the treatments, relative to untreated control, the two phytonematicides and Velum (a.i. fluopyram) significantly increased plant growth, fruit yield and quality, although with the accumulation of phosphorus in leaf tissues, with efficacies of the three products being comparable. Similarly, relative to untreated control, the three products significantly reduced nematode eggs and juveniles in roots and juveniles in soil, with efficacies that were significantly comparable. In conclusion, the benefits of phytonematicides on the productivity of watermelon cv. 'Congo' and suppression of population densities of *M. enterolobii* were comparable.

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Introduction

Watermelon (*Citrullus lanatus* Thunb.) cultivars do not have genotypes with resistance to root-knot (*Meloidogyne* species) nematodes. Yield losses on watermelon due to infection by *Meloidogyne* species are from as high as 50% to complete crop failure (Thies and Levi 2007; Thies et al. 2009). Prior to the 2005 withdrawal of fumigant chemical nematicides from the global agrochemical markets, methyl bromide was widely used in managing nematode population densities in watermelon production (Thies et al. 2009). The systemic carbamates and organophosphates were not preferred in watermelon production due to incidents of chemical residues in fruit, which at times resulted in consumer fatalities (Thies et al. 2009). In watermelon, aldicarb metabolite residue, aldicarb sulfoxide, from 1985 to 1988, at concentrations near the lowest detection level of 0.2 ppm, poisoned more than a thousand people in the U.S.A. (Goldman et al. 1990). In other parts of the world, where organophosphate and organochlorine chemical nematicides were used, the maximum residue limits were awesomely above those set by the WHO/FAO (Essumang et al. 2017).

The use of nematode-resistant rootstocks from within the Cucurbitaceae family outside the genus *Citrullus*, technically referred to as intergeneric grafting, had been widely investigated as an alternative to fumigant chemical nematicides (Thies and Levi 2007; Thies et al. 2009; Pofu et al. 2012; Liu et al. 2015). In *Cucumis-Citrullus* intergeneric grafting, in addition to suppressing nematode population densities, watermelon flowered earlier and accumulated large quantities of certain essential nutrient elements in leaf tissues (Pofu et al. 2012). However, the grafting technique was labour intensive, with resistance being inconsistence due to the existence of races within *Meloidogyne* species. Races are morphologically similar within a given species and were historically identified using differential

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host plants (Taylor and Sasser 1978), with molecular data based on 18S rDNA and ITS rDNA of nematodes being the modern tool of choice (Floyd et al. 2002; Blaxter 2004; Powers 2004). Wild cucumber (*Cucumis myriocarpus* Naude.) and wild watermelon (*Cucumis africanus* L.) indigenous to South Africa were highly resistant to South African isolates of *M. incognita* and *M. javanica* (Pofu et al. 2012), but in China, only *C. africanus* was highly resistant to the test *M. incognita* isolate, whereas *C. myriocarpus* was moderately resistant to the isolate (Liu et al. 2015).

Historically, *M. incognita* was viewed as the most widely distributed thermophilic *Meloidogyne* species, with the status of being more aggressive than *M. javanica* (Taylor and Sasser 1978). However, in South Africa, population densities of the two species occurred predominantly as mixed populations, with *M. javanica* being more aggressive than *M. incognita* isolates (Kleynhans et al. 1996). Currently, another thermophilic *Meloidogyne* species, *M. enterolobii* (Yang and Eisenback 1983), with ontogenies of 15 days (Collet 2020), is emerging as the most aggressive, with yield losses being from as high as 65% to complete crop failure (Castillo and Castagnone-Sereno 2020; Philbrick et al. 2020). Due to its wide host range and the ability to reproduce on tomato genotypes with Mi resistance genes, *M. enterolobii* has gained the global status of an emerging threat in various crop production systems (Philbrick et al. 2020). After observing that the long-term crop rotation systems that we were evaluating were failing to contain nematode population densities for the successor nematode-susceptible crops, molecular techniques suggested that instead of mixed *Meloidogyne* species, the fields were predominantly infected with *M. enterolobii* (Chiuta 2021; Maleka 2021).

The desired nematode management strategy in cropping systems should not be species-specific or race-specific in reducing the population densities of *Meloidogyne* species. In addition to being cost-effective, the strategy should be free of challenges associated with chemical synthetic nematicides (Van Gundy 1987; Goldman et al. 1990; Essumang et al. 2017). Two cucurbitacin phytonematicides, developed from fruits of wild *Cucumis* species, *C. myriocarpus* (Nemarioc-AL phytonematicide) and *C. africanus* (Nemafric-BL phytonematicide) were developed to meet the requirements of such a strategy. The active ingredients cucurbitacin A ($C_{32}H_{46}O_9$) and cucurbitacin B ($C_{32}H_{46}O_8$) in the two respective phytonematicides are primarily non-polar (Chen et al. 2005). Such non-polar molecules cannot move from soil solution into the vascular bundle of plant roots, *vice versa* (Van Wyk and Wink 2004), due to the presence of the pericycle and the endodermis in most plant roots, which confer symplastic barriers. The two phytonematicides did not leave any cucurbitacin residues in fruit of tomato plants (Dube 2016; Shadung 2016). Another product, introduced to the agrochemical markets as a fungicide/insecticide – Velum, is currently being used as a nematicide in various cropping systems. However, the cost of the product is prohibitive, especially in packages intended for smallholder farming systems. The efficacy of cucurbitacin phytonematicides and Velum on the productivity of watermelon and suppression of nematode population densities on the crop had not been documented. The objective of this study was therefore to investigate the comparative efficacies of the two cucurbitacin Nemarioc-AL phytonematicide, Nemafric-BL phytonematicide and Velum on the productivity of watermelon cv. 'Congo', its accumulation of essential nutrient elements in leaf tissues and suppression of *M. enterolobii* population densities under field conditions.

Materials and methods

Description of study location and land preparation

The study was conducted during mid-summer (Nov 2019) and validated in 2020 at the Green Biotechnologies Research Centre of Excellence, University of Limpopo, South Africa (S23°53'10" E29°44'15"). The location has semi-arid climate, with rainfall skewed towards summer months, with predominantly loamy soil (65% sand, 30% clay, 5% silt). Primary land preparation was achieved using a mouldboard plough and levelled using hand rakes. A drip irrigation system was laid out to allow for 2 L water/drip hole/h at 0.90 m × 1.2 m spacing. Soil samples were collected for initial nematode population densities (Pi) using a soil sampler probe 52 cm to collect 10 cores per plot, with nematode second-stage juveniles (J2) extracted from 250 ml soil subsample from each plot using the modified sugar floatation centrifugation method (Marais et al. 2017). Watermelon cv. 'Congo' seeds were primed in tapwater for six hours and then sown in 200-hole seedling trays containing Hygromix-T growing mixture (Hygrotech, Pretoria North) and then placed on greenhouse benches. At four-leaf stage after emergence, seedlings were hardened-off for 14 days through intermittent withdrawal of irrigation water.

Treatments, experimental design and procedures

Prior to transplanting, each planting station was irrigated daily for 2 weeks for a total of 600 mm water

and then after transplanting by 25 mm weekly until harvest. Since Pi was low (Pi = 9 J2, range 0–30), at transplanting each seedling was further inoculated with 250 eggs + J2 by placing in holes around the stem using a 15 ml plastic syringe, with holes filled with soil. Treatments, comprising Nemafric-BL phytonematicide, Nemarioc-AL phytonematicide, Velum and untreated control, were arranged in a randomised complete block design, with 12 replications. Treatments were initiated at seven days after transplanting and applied biweekly at 2% phytonematicide per seedling using 500 ml chlorine-free tapwater, while Velum was applied once using 500 ml solution at 0.08 ml/15 L chlorine-free tapwater as per label instruction.

Cultural practices

Fertilisation at transplanting comprised 5 g 2:3:2 (22) N:P:K fertiliser mixture and 5 g superphosphate (10.6%), each applied at 5 cm away from the stem of seedlings. The first top dressing was done at 4 weeks after transplanting using 5 g Lime ammonium nitrate (LAN) and 5 g 2:3:4 (30) N:P:K fertiliser mixture, which were applied separately in holes around the stem and covered with soil. The second top-dressing was applied using 5 g LAN and 5 g potassium nitrate in holes around the stem at six weeks after transplanting. Potential damage by fruit-fly (*Bactrocera dorsalis* Hendel 1912) was managed by three sprays of Malathion 25 EC at 25 ml/L water at 15-day interval from flowering. Additionally, a weekly preventative spraying programme comprising alternating Mancozeb, copper oxychloride and Bravo as per label instruction, was designed to manage incidents of late blight, early blight, anthracnose, downy mildew and powdery mildew. Weeds were controlled using hand-hoes when the transplants were still young and thereafter manually pulled out when necessary.

Data collection and analysis

At harvest, 90 days after transplanting, marketable fruit were harvested and weighed. Degrees Brix (°Bx) was quantified using a hand-held refractometer (Bellingham and Stanley, UK). Plant length was measured from the crown to the tip of the longest runner, shoots were cut at the soil level and stem diameter measured at 5 cm above the severed ends using a digital Vernier caliper. Ten mature and healthy leaves were collected per plant, rinsed in distilled water, with excess water removed by pressing between laboratory paper towel, along with shoots dried at 60°C for 72 h and weighed. Dried leaves were ground using the Thomas Model 4 Wiley Mill, with 0.4 g powdered material subjected to the digestion method (Zygmunt and Namiesnik 2003). Digested samples were quantified for selected essential nutrient elements using the Atomic Absorption Spectrophotometer ICPE-9000 (Jones and Case 1990).

Root samples were collected, immersed in water to remove soil particles and blotted dry using a laboratory paper towel. Approximately 10 g root was used for extracting eggs and J2 using the modified maceration and blending method for 30 s in 1% NaOCl solution (Marais et al. 2017). The aliquot was passed through top-down nested 45-µm and 25-µm mesh sieves. Contents of the 25-µm mesh sieve were poured into 100-ml plastic containers for counting under a stereomicroscope. J2 were extracted from 250 ml soil subsample using the modified sugar-floatation and centrifugation method (Marais et al. 2017).

Fruit number and nematode (eggs, J2, Pf) were transformed using $\log_{10}(x + 1)$, with each dataset subjected to the Shapiro–Wilk test to determine the normality of the distribution of data (Shapiro and Wilk 1965; Ghasemi and Zahediasl 2012). The data were normally distributed and therefore, were subjected to analysis of variance using Statistix 10.0 software. Treatment efficacies were compared at the probability level of 5% using the Tukey HSD All-pairwise Comparison test. Unless otherwise stated, only treatment effects which were significant at 5% level of probability were discussed.

Results

Seasonal interactions were not significant and therefore, data were pooled and re-subjected to ANOVA ($n = 96$). Treatment effects were significant on dry shoot mass, fruit number, fresh fruit mass, vine length and degrees brix (°B), but had no significant effects on stem diameter. Relative to untreated control, Velum, Nemafric-BL phytonematicide and Nemarioc-AL phytonematicide increased fruit number by 236, 254 and 133%, fresh fruit mass by 185, 68 and 44%, along with vine length by 32, 48 and 29%, respectively (Table 1). However, in fruit number and fresh fruit mass, the effects of Velum and the two phytonematicides were not different ($P \leq 0.05$) from one another. Nemafric-BL phytonematicide resulted in the longest vine length when compared with untreated control, which was, however, not different to that of Nemarioc-AL phytonematicide and Velum. Similarly, relative to untreated control, Nemarioc-AL phytonematicide resulted in significantly higher total soluble solids (TSS) in watermelon fruit (RI = 20%), but the effects were comparable to those of Nemafric-BL phytonematicide and Velum.

Table 1. Relative impact (RI) of Velum, Nemafric-BL (BL) and Nemarioc-AL (AL) phytonematicides on dry shoot mass (DSM), fruit number (FN), fresh fruit mass (FFM) vine length (VL) and degrees brix (°B) of watermelon cultivar 'Congo' under field conditions at 90 days after transplanting.

Treatment	DSM (g/plant)	RI (%)	FN[z]/plant	RI (%)	FFM (g/plant)	RI (%)	VL (cm/plant)	RI (%)	TSS (°B)	RI (%)
Control	19.23	–	0.483^{b}	–	1272.0^{b}	–	42.9^{b}	–	19.1^{b}	–
Velum	20.20	5	1.625^{a}	236	2350.4^{a}	185	56.5^{ab}	32	20.3^{b}	6
BL	21.03	9	1.708^{a}	254	2131.4^{a}	68	63.7^{a}	48	22.9^{a}	17
AL	19.75	5	1.125^{a}	133	1824.7^{a}	44	55.4^{ab}	29	20.70^{b}	20

[z]Column means followed by the same letter were not different ($P \leq 0.05$) according to Tukey test.

The treatments significantly affected P in leaf tissues of cv. 'Congo'. Relative to untreated control, Nemarioc-AL and Nemafric-BL phytonematicides increased P in leaf tissues of watermelon by 34 and 13%, respectively. However, the effects of Nemafric-BL phytonematicide and Velum on P were comparable (Table 2). The treatments did not have significant effects on Ca, Mg, K, Mn, Na, Fe and Zn in leaf tissues of the test plant.

Treatment effects on J2 of *M. enterolobii* in soil, eggs in root, J2 in root and total nematode population density were highly significant, contributing 78, 60, 73, and 69% in total treatment variation (TTV) of the respective variables (Table 3). Relative to untreated control, Velum, Nemarioc-AL phytonematicide and Nemafric-BL phytonematicide reduced the four respective variables by 93, 86, 79 and 90%, which were not significantly different from one another. Roots of untreated control plants were heavily galled, with J2 in roots averaging 618, range 43–927 (Data not shown).

Discussion

The comparable efficacies of cucurbitacin phytonematicides to those of Velum on growth of watermelon (Table 1), agreed with those of the products on growth of potato (*Solanum tuberosum* L.) plants (Seshweni 2017). Nemarioc-AL phytonematicide had similar comparative efficacies with aldicarb and fenamiphos on growth of tomato (*Solanum lycopersicum* L.) plants (Mashela et al. 2008). Generally, when cucurbitacin phytonematicides are applied at an empirically-derived concentration within the 2-3% range, the products invariably stimulate plant growth (Mashela et al. 2017). The phenomenon was previously referred to as a 'fertiliser effect', although nutrient elements in leaf tissues of treated and untreated plants did not differ (Mashela 2002). Relative to untreated control, all the test products in the current study stimulated growth of watermelon cv. 'Congo', which agreed with observations in potato production (Seshweni 2017). Generally, infection of plants by *M. incognita* and *M. javanica* each reduced stem diameter (Mashela 2002, 2017). However, the effect was not observed in watermelon plants infected by *M. enterolobii,* especially on plants under untreated control.

The significant increase of °B by Nemafric-BL phytonematicide (Table 1) was consistent with improvement of °B by cucurbitacin phytonematicides in sweet stem

Table 2. Relative impact of Velum, Nemafric-BL and Nemarioc-AL phytonematicides to accumulation of selected nutrient elements (ppm) in leaf tissues of cultivar 'Congo' at 90 days after transplanting.

Treatment	Ca	P[z]		Mg	K	Mn	Na	Fe	Zn
Control	32.128	5.176^{c}	–	26.079	37.562	0.448	8.407	16.643	7.530
Velum	34.117	5.513^{bc}	7	25.317	39.013	0.672	8.032	18.767	8.203
Nemafric-BL	30.628	5.865^{b}	13	26.050	40.125	0.412	9.713	17.314	6.801
Nemarioc-AL	30.242	6.927^{a}	34	25.208	44.654	0.384	8.325	18.397	7.165
$LSD_{0.05}$	9.546	–	–	1.882	5.922	0.449	1.494	3.320	4.090

[z]Column means followed by the same letter were not different ($P \leq 0.05$) according to Tukey HSD All-pairwise Comparison test.

Table 3. Partitioning of sources of variation in second-stage juveniles (J2), eggs and total nematodes (Pf) from watermelon cultivar 'Congo' under control, Velum and the two-cucurbitacin phytonematicides at 90 days after transplanting.

		J2 in soil		J2 in root		Eggs in root		Eggs + J2 in root		Pf	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Rep	23	7.923	21	9.397	37	2.092	23	0.129	22	18.325	30
Trt	3	29.692	78***	15.128	60***	6.771	73***	0.417	23**	41.981	69***
Error	69	0.312	1	0.081	3	0.384	4	0.232	4	0.738	1
Total	95	37.927	100	25.332	100	9.247	100	0.569	100	61.044	100

TTV = Total treatment variation.

sorghum under field conditions (Mashela and Pofu 2016; Maleka 2021). Although the mechanism through which cucurbitacin phytonematicides improve °B in produce of certain plants is not yet understood. However, as observed in various sweet stem sorghum experiments (Maleka 2021), it appears that, this phenomenon as induced by cucurbitacin phytonematicides is consistent in crops and should therefore, be investigated further.

The influence of Nemafric-BL and Nemarioc-AL phytonematicides on P in leaf tissues of watermelon in the current study (Table 2), confirmed observations on watermelon cultivars (Nhlane 2017), tomato plants (Maake 2018), green beans (Mashela and Pofu 2017) and cowpea (Mashela 2014). Generally, when crops were subjected to increasing concentration of cucurbitacin phytonematicides, P in leaf tissues versus phytonematicides exhibited positive quadratic relations, whereas other elements had either positive or negative quadratic relations or no relations at all (Mashela et al. 2017). Phosphorus is used in various physiological activities, such as protein and nucleoprotein biosynthesis, and in metabolic transfer processes such as adenosine diphosphate and adenosine triphosphate during photosynthesis and respiration, respectively. Notably, cucurbitacin phytonematicides have no effect on soil pH, which is instrumental in the availability of soil P to plants.

Similarities on the efficacy of Velum to cucurbitacin phytonematicides on suppression of various stages of *M. enterolobii* in soil and in roots (Table 3), confirmed consistent suppressive effects of the two phytonematicides in different cropping systems (Mashela et al. 2017). Findings in the current study confirmed comparative efficacies of the two phytonematicides on suppressive effects on nematode population densities of *Meloidogyne* species when compared with those of Velum on potato plants (Seshweni 2017) and those of aldicarb and fenamiphos on tomato plants (Mashela et al. 2008). Notably, active saponins from alfalfa (*Medicago sativa* L.) were previously shown to reduce population densities of *M. incognita* significantly more than fenamiphos (D'Addabbo et al. 2010). Basically, carbamates such as aldicarb and organophosphates such as fenamiphos had nematostatic effects on nematode J2 (Van Gundy and Mc Kenry 1975; Goldman et al. 1990), whereas the cucurbitacin phytonematicides have nematicidal effects which include total disintegration of nematode proteins (Mashela and Shokoohi 2021).

Conclusion

Cucurbitacin phytonematicides and Velum had stimulation effects on growth of watermelon, with the potential of improving biomass, accumulation of P in leaf tissues, fruit yield and quality. The efficacies of the test phytonematicides were comparable to that of Velum in suppression of population density of *M. enterolobii* on watermelon under field conditions.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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