Control of root-burrowing nematode (*Radopholus similis*) in banana using extracts of *Azadirachta indica* and *Allium sativum*

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Abstract

The root-burrowing nematode, *Radopholus similis* is considered to be the most destructive nematode associated with banana production worldwide. This nematode can reduce plant growth and yield by more than 50% and decrease the productive life of banana fields. *R. similis* control in the Windward Islands banana industry has been based on the application of synthetic nematicides, which are now prohibited due to human health and environment hazards. One possible alternative is the utilisation of plant extracts with known nematicidal effects such as *Azadirachta indica* and *Allium sativum*. The efficacy of these phytochemicals at managing *R. similis* were assessed and compared with a synthetic nematicide, ethoprophos (Mocap 15G) in two banana pot trials. There were five treatments (Control A, Control B, Neem X, Garland, and Mocap 15G) with 6 plants per treatment. The results of the root and soil extractions showed that all treatments caused a significant reduction in *R. similis* population density (P = 0.05), with Mocap 15G being the most effective. From the plant growth data it was observed that all treatments caused a significant increase in plant growth (P = 0.05). No significant difference in the pseudostem length and girth, as well as, the leaf number and area were observed between treatments. The root and corm weights also showed no significant differences between treatments. The efficacy of botanical nematicides and their effect on banana production in the Windward Islands are discussed.

Keywords: allicin, azadirachtin, botanical control, nematostat, Trinidad and Tobago.

Introduction

The banana industry has been a key source of employment and foreign revenue to the Windward Islands for over eighty years (NERA, 2004). The banana trade has provided a direct living for thousands of small-scale producers and has accounted for up to 50% of the Windward Islands' total export revenue with sales of 274,000 tonnes a year to a value of US$147m. This industry, while blessed with a warm tropical climate, is threatened by tropical storms and a complex of fungi, insects and nematodes, the latter being one of the most important limiting factors. These factors have led to a major reduction in banana production and exports to a mere 99,000 tonnes a year at a value of US$45m (Wiltshir, 2004). The root-burrowing nematode, *Radopholus similis* (Cobb 1893) Thorne 1949 is considered to be the most destructive nematode associated with banana production worldwide (Gowen et al., 2005). *Radopholus similis* feeds on the cortical cells of root and corm (rhizome) tissues causing cavities to develop, which evolves as root necrosis (Brooks, 2008). This in turn reduces growth and yield by more than 50%, lengthens the time to fruiting, and decreases the productive life of banana fields (Quénéhervé et al., 2006). Plant anchorage is also affected, which results in toppling or uprooting (Gowen et al., 2005) Effective *R. similis* control is therefore essential for the survival of the banana industry.

The uptake of organic principles of production has been slow in Trinidad and Tobago. Although 164 countries report certified organic agriculture statistics, and some report organic banana production, no organically managed land is reported by Trinidad and Tobago (Willer & Lernoud, 2014). In the past, in Trinidad and Tobago, *Radopholus similis* control has been based mainly on the use of synthetic nematicides such as, ethoprophos, oxamyl and aldicarb (Chabrier & Queneherve, 2003). These products are now prohibited due to their adverse impacts on human health and the environment (Nagaraju et al., 2010). Windward Islands banana farmers are also barred from using other synthetic nematicides due to Fair-trade arrangements (Isaac et al., 2007). Plant-based pest control agents have long been touted as alternatives to synthetic nematicides (Javed et al., 2006). Such phytochemicals reputedly pose little threat to the environment or to human health and their use is approved under organic and Fair-trade arrangements (Amadioha, 2003). However, the adoption of such a management strategy has been met with scepticism by banana producers who question the efficacy and consistency of these phytochemicals (Villanueva 2005). In this study, phytochemicals derived from *Azadirachta indica* and *Allium sativum* extracts were assessed and compared with ethoprophos for their effectiveness at reducing *R. similis* population density in the roots and soil, and preventing banana growth and yield losses.

Materials and methods

Two pot trials were conducted throughout 2009 and 2010 at the University of the West Indies Field Station (UFS) situated in Valsayn, Trinidad (10° 39' 0" N, 61° 25' 0" W). The average monthly temperature and precipitation at UFS were 27.2℃ and 1720 mm, respectively for the length of the study. The growing medium comprised of sterilised Fluventic Eutropept soil (River Estate Loam), with a cation exchange capacity of 4.8 cmol/kg and a pH of 6.5.

Experimental design

Each trial consisted of 30 banana plants (cv. Lacatan), planted in 50 L plastic-drums and spaced 2×2m apart. The drums were arranged in a completely randomised design with five treatments: Control A, Control B, Neem X® (Marketing Arm International), Garland® (OMEX Agriculture Ltd) and Mocap 15G® (Bayer Crop Science) and six replicates. Plants were grown for 1 month and then inoculated with 500 *R. similis* following the procedures of Speijer & De Waele (1997). One month after inoculation, nematicidal treatments were applied as a soil drench (1L) at the base of the pseudostem. The active ingredients, sources, application rates and frequencies of each treatment are given in Table 1. Agronomic management was done according to the protocols recommended in the Windward Islands for banana production, which included fertilisation, irrigation, pruning, propping, de-suckering, and pest and disease management (Paul et al., 1993).

Table 1. List of treatments with active Ingredients and application rate and frequency

Treatments	Active Ingredients	Application rate	Application frequency
Control A	No *R. similis*	-	-
Control B	*R. similis* only	-	-
Neem X®	Azadirachtin (3000 ppm)	2.7ml/L	7 days intervals for 3 applications
Garland®	Allicin (<1 ppm)	2.16ml/L	7 days intervals for 3 applications
Mocap 15G®	Ethoprophos	15g/m^2	7 days intervals for 3 applications

Data collection

***Radopholus similis* population density**

An estimation of the *R. similis* population density was done using extraction methodologies described by Southey (1986) for the roots, and Whitehead & Hemming (1965) for the soil. The blender nematode filter extraction method was used to extract nematodes from 15g of roots while the Whitehead tray method was modified to extract nematodes from 200ml of soil. The collected nematode sample was identified and counted in three 1ml aliquots out of a 10ml aqueous suspension using a stereoscopic microscope. All vermiform stages (juveniles and adults) were counted.

Root necrosis index and banana root and corm fresh weight

At the end of each trial the plants were excavated, their roots and corms cleaned to remove soil particles and their root necrosis index (RNI) and root and corm fresh weights (RCFW) determined. A modified Bridge & Gowen (1993) root necrosis index was used to determine root rotting on a 0 to 4 scale [0 = no damage; 1 = <25% of total root cortex with necrosis; 2= 26–50% of the total root cortex with necrosis; 3 = 51–75% of total root cortex with necrosis; 4 = >75% of total root cortex with necrosis]. Fresh weight (kg) was determined using a Rebure pocket balance (Germany).

Plant growth

Throughout the study the following crop measurements were recorded weekly:

(a) The pseudostem length (cm) measured from the point of the lowest leaf to the base of the pseudostem.
(b) The pseudostem girth/circumference (cm) measured from a point at half the pseudostem length.
(c) The total number of fully opened functional leaves (Fogain, 2000).
(d) Leaf area (LA) predicted with the regression models: LA=0.0266+(L×W×0.7629) (r = 0.98), where L= leaf length and W= leaf width. The third leaf from the top of the plants was selected as the standard leaf for measurement (Potder & Pawer, 1990).

Data Analysis
Differences in nematode density, necrosis index, roots and corm fresh weight, and growth parameters were analysed with the use of analysis of variance (ANOVA). Pearson correlation was used to determine the strength of relationship between the *R. similis* density in the roots and the necrosis index. Prior to analyses, variables were tested for homogeneity of variances and normality, and data found to be non-homogenous were either $\log_{10}$ (X + 1) or square root transformed before statistical analysis. Non-transformed means were reported in Figures and Tables and only significant differences ($P \leq 0.05$) are discussed unless stated otherwise. All statistical analysis was performed using the statistical software Minitab® 16.1.1 (Minitab Inc).

Results and Discussion

Radopholus similis *population density*
All nematicides were successful at reducing *R. similis* population density in the root and soil. Plants treated with ethoprophos had a lower *R. similis* density than those treated with *Azadirachta indica* and *A. sativum* extracts (Figure 1). Several studies have reported on the notable effects of ethoprophos in *Musa* spp. which acts as a nematostat in low concentrations (Stirling & Pattison, 2008; Quénéhervé, 2009; Radwan et al., 2012). However, to maintain its high effectiveness, frequent applications were required, which increases its negative effects on the environment and human health (Sipes & Schmitt, 1995).

Plants treated with *Azadirachta indica* had a high *R. similis* density in the soil (570) but a lower density in roots (275). This disparity was due the anti-feedant properties of azadirachtin, the phytochemical found in *Azadirachta indica* (Sidhu, 2003). Rehma et al., (2009) suggested that azadirachtin can induce nematostatis, a process that inhibits nematodes from invading plants without directly killing them. The phytochemicals found in *Allium sativum* (allicin) demonstrated a similar but less effective anti-feedant effect and was inconsistent at reducing *R. similis* density in the roots. This inconsistency may be due to the life cycle of *R. similis*, which can be completed in the root, without any stage in the soil. Thus preventing exposure to the allicin applied in the soil (Araya, 2003).

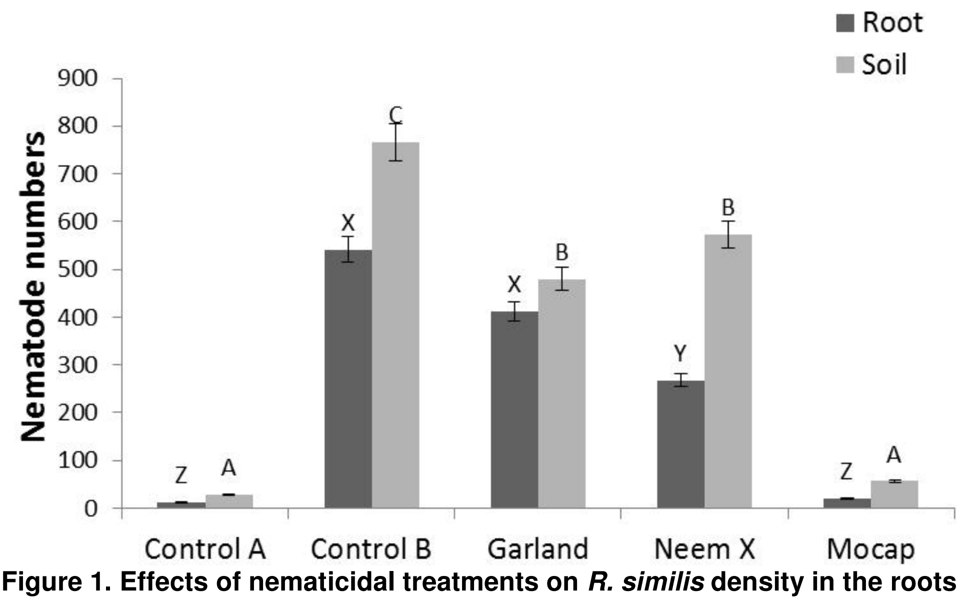

Figure 1. Effects of nematicidal treatments on *R. similis* density in the roots and soil of banana plants. Values are the average of 6 replicates. Bars with the same letter are not significantly different (*P* > 0.05).

Endo-parasitic nematodes, such as *R. similis* were expected to be more prevalent in the roots than the soil. This study showed a deviating trend which can be attributed to the nematostatic activities of the treatments along with the inefficiencies of Southey's (1986) extraction technique at recovering *R. similis* from the roots. This is a major deficiency in Southey's technique since endo-parasitic nematodes were unlikely to migrate from healthy/necrosis free root tissues to be extracted by this technique. Therefore, the estimated nematode density in the roots may have been lower than the actual nematode density.

Root necrosis index

The differences in the RNI between treatments were statistically significant (P < 0.05) in both trials, with Control A having the lowest index value and Control B the highest (Table 2). The Pearson correlation between *R. similis* density and the root necrosis were positive in both trials (Trial 1: R^2 = 0.574; Trial 2: R^2 = 0.190). Therefore, increases in the *R. similis* density in the roots will result in an increase in the root necrosis. The strength of the relationship was however inconsistent and the weak correlation in Trial 2 supports the assumption that highly necrotised root tissue may have a lower *R. similis* density. Dosselaere (2003) indicated that nematodes may move out of resource scarce necrotic banana root tissue and reinvaded healthy tissue. Plants treated with *Azadirachta indica* had the lowest root necrosis index in both trials due to the nematostatic activities of azadirachtin, which inhibits nematodes invasion into the roots. *Allium sativum* had similar but less effective nematostatic activities, while the efficacy of ethoprophos was inconsistent.

Table 2. Mean roots necrosis index values and root and corm fresh weight (kg).

	Root necrosis index		Root and corm weight (kg)	
Treatments	Trial 1 (SD)	Trial 2 (SD)	Trial 1 (SD)	Trial 2 (SD)
Control A	1.33 (0.52)	1.83 (0.75)	2.2 a (0.75)	2.1 a (1.11)
Control B	2.67 (0.75)	2.17 (0.75)	1.4 b (0.58)	1.6 b (0.80)
Garland	2.17 (0.82)	1.83 (0.98)	1.6 b (0.60)	2.2 a (1.24)
Neem-X	1.83 (0.75)	1.67 (0.82)	1.8 ab (0.71)	2.1 a (0.56)
Mocap	1.83 (0.98)	2.17 (0.98)	2.0 a (0.33)	2.1 a (1.14)

Banana root and corm fresh weight

All treatments were effective at maintaining healthy root and corm. Ethoprophos performed consistently in both trials and recorded the highest RCFW among the treatments. The anti-feedant effect of azadirachtin and allicin were also consistent and comparable to ethoprophos. *Radopholus similis* population densities in the roots and corm tissues had a direct effect on its RCFW. Moens et al. (2003) found a linear reduction of root weight when *R. similis* was inoculated at increasing densities. Marin et al. (1999) and Sarah (2000) also found decreases in root weight, ranging from 8-80%, several weeks after inoculation with *R. similis*. The data obtained in this study were similar to those reported in the literature, as Control A had the highest sum of RCFW while Control B had the lowest (Table 2). Therefore, RCFW shows some potential for used as a rapid technique for estimating *R. similis* density (Moens et al 2003). However, depending solely on RCFW to determine the implementation of nematode control strategies may not be advisable, as root and corm weight may vary due to factors other than nematode density, such as the banana weevil borer (*Cosmopolites sordidus*) and plant nutrient (Sarah, 2000).

Plant growth

The banana growth data confirmed that the phytochemical treatments were comparable to ethoprophos at preventing plant growth losses, as no significant differences were observed in both trials (Table 3). This may also be due to the ability of banana cv. Lacatan to withstand *R. similis* infestation. Therefore, plant sensitivity or tolerance must be considered since nematode tolerance has been identified in the *Musa* gene pool (Dochez et al., 2006) The selection of nematode tolerant banana cultivars can play a very important role in nematode management and is ranked as one of the ideotype requirements for the acceptation of banana hybrids (Tenkauano & Swennen, 2004). On the other hand, a tolerant but susceptible banana plant is of limited value, as nematode reproduction may increase the population density beyond the damage threshold (Cook & Starr 2006). Therefore, in the absence of true resistance, incorporating tolerant plants with phytochemicals may be an effective strategy for preventing the *R. similis* density from crossing the plant damage threshold and causing yield losses.

Table 3. Effects of the treatments on the growth parameters of banana plant.

	Pseudostem		Leaf	
	Length (cm)	Girth (cm)	Numbers	Area (cm^2)
Control A	95 (0.52)	28.0 (0.75)	6.0 (0.75)	2,673 (1.11)
Control B	77 (0.75)	23.6 (0.75)	7.5 (0.58)	2,182 (0.80)
Garland	82 (0.82)	26.2 (0.98)	6.7 (0.60)	2,363 (1.24)
Neem-X	85 (0.75)	27.5 (0.82)	6.7 (0.71)	2,498 (0.56)
Mocap	92 (0.98)	28.5 (0.98)	6.5 (0.33)	2,691 (1.14)

Conclusions

The application of phytochemicals (azadirachtin and allicin) as alternatives to synthetic nematicides was effective and comparable to ethoprophos at preventing plant growth and yield losses. This will be beneficial to banana producers by decreasing the time for fruiting and increasing the productive life of the fields. Plants treated with azadirachtin also had a low root necrosis index which may result in fewer plants toppling and uprooting. However, the phytochemicals were less effective at reducing *R. similis* density in the soil which leaves the plants vulnerable to future infestations unless other nematode management strategies are adopted.

In the pursuit of more benign pest management and more consumer-acceptable solutions in banana production it would be appropriate for more research to be directed to organic research and some attention paid to the lessons learnt in other countries which are pursuing the expansion of their own organic banana production.

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